Review and Research on Cancer Treatment
Volume 9, Issue 1 (2023)

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Volume 9, Issue 1 (2023)

ISSN 2544-2147

Publisher:
Fundacja na rzecz promocji nauki i rozwoju TYGIEL
ul. Głowackiego 35/348, 20-060 Lublin
www.fundacja-tygiel.pl

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Proteostasis and Aggresomes – as possible target for cancer therapy

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ABSTRACT

Efficient protein metabolism is essential to maintain intracellular homeostasis. Especially when the concentration of misfolded proteins increases. Cells evolve complex mechanisms to regulate protein synthesis, refolding, and degradation. There are two main mechanisms of protein degradation: the ubiquitin-proteasome system (UPS) is responsible for the degradation of small soluble proteins, and autophagy associated with hydrolytic lysosomal enzymes is responsible for the degradation of larger insoluble structures. In the process of carcinogenesis, genomic instability increases, and control mechanisms become insufficient. As a result, these two degradation pathways become insufficient, and the concentration of abnormal proteins increases. This favors the formation of aggresomes – the pericentriolar aggregates containing mostly insoluble proteins. Many studies confirm the cytoprotective nature of these structures.

Understanding the basis of molecular disorders in the cancer cell is crucial for developing novel therapies. Drugs affecting protein turnover induce endoplasmic reticulum stress. They also act preferentially to the cancer cells due to increased protein production. Proteasome inhibitors (PIs) – bortezomib, carfilzomib, and ixazomib – have been used to treat multiple myeloma and mantle cell lymphoma. PIs also act on numerous signaling pathways. In addition, immune-selective PIs are under evaluation in treating auto-immune diseases. Panobinostat – an inhibitor of histone deacetylases, including HDAC6, the regulator of transport to the aggresome, is also used in treating multiple myeloma. More research on drugs affecting proteostasis is needed to gain new therapeutic possibilities. Newly synthesized drugs enable more options in cancer therapy and the treatment of other diseases.

INTRODUCTION

Cellular metabolism and architecture complexity require constantly flowing newly synthesized proteins through the cell lifespan. An eukaryotic cell contains from 100 million to 100 billion molecules of proteins (Harper, 2016). For management of such huge number of components, cells develop a precise system to regulate protein synthesis and degradation. Similarly, the newly synthesized linear amino acid polymer becomes folded to the correct three-dimensional structure and is transported to a defined subcellular space to fulfill its function. If any stage of this mechanism fails, misfolded and incorrectly localized polypeptides disrupt other cellular processes (Chiti, 2017). Protein folding failure may occur due to changes in amino acid sequence resulting from DNA mutations, errors in the transcription and translation, or imbalance in protein synthesis (Bonifacino, 1989).

On the other hand, environmental stressors such as temperature, osmotic pressure, oxidative stress, or viral infections can also disturb protein folding (Johnston, 1998). Moreover, by exposure of hydrophobic domains, misfolded or denatured proteins may aggregate into toxic polymers (Garcia-Mata, 1999). Therefore, evolution equips cells with mechanisms that help them to manage unfolded or damaged proteins.

The system that is responsible for maintaining of protein homeostasis is called the proteostasis network. That system may move proteins with nonnative conformations to the pathways of refolding, degradation, or formation of aggregates. Indeed, years of research on cell biology revealed that these three mechanisms are closely related, complement each other, and many enzymes participate in two or all patterns (Johnston, 2021). Proteotoxicity leads to compensatory mechanisms of cellular response by reducing global protein transcription, producing proteostasis network components, and recruiting present ones. Misfolded proteins, primarily by recognition of presenting hydrophobic domains, are bonded by protein folding enzymes – molecular chaperones. Among many molecular chaperones, stress-induced heat-shock proteins 70 and 90 (HSP70, HSP90) are essential in response to proteotoxic stress. They rearrange damaged proteins and assist in the folding of *de novo* translated polypeptides, which are the most vulnerable to stress-induced misfolding (Jayaraj, 2020). Conversely, failure of protein refolding directs them to the degradation pathway. Also, an overload of chaperones' capacity and an insufficient supply of amino acids push proteins into the clearance system.

We can distinguish two main clearance pathways: the ubiquitin-proteasome system (UPS) and system associated with autophagy and involving hydrolytic lysosomal enzymes. UPS mainly degrades relatively small soluble proteins, while larger insoluble macromolecules and organelles are surrounded and degraded

by the autophagosome. These two pathways exist pararelly and complement each other. During endoplasmic reticulum (ER) stress proteins are retrotranslocated from the ER into the cytosol and exposed to degradation via UPS. When the amount of misfolded protein in ER overwhelms cellular transport capacity to cytosol, autophagy is induced to handle protein overload (Almanza, 2019).

USP – ON GUARD OF PROTEOSTASIS

The USP remains the most exploited cellular clearance pathway since it is ubiquitous and present in every eukaryotic cell in both cytosol and in nucleoplasm. UPS degrades over 80% of all cytoplasmic proteins (Glickman, 2002). Despite the clearance function, degradation via USP participates in various biological processes such as cell cycle regulation, signal transduction, and gene transcription (Wojcik, 2002).

The crucial mediator of UPS degradation and an essential label in protein trafficking is small globular polypeptide, ubiquitin (Ub) – it contains 76-amino acids and weights 7 kDa. For degradation by UPS at least four Ub has to be attached to the protein substrate. This highly conservative polypeptide is covalently attached by C-terminal glycine to lysine residues in protein structure in the ubiquitination process (Haglund, 2005). Although, recent reports confirm that, Ub could be linked to threonine, serine, and cysteine too (cytowanie). It is worth emphasizing that lysine – substrate bond in ubiquitin is the most intensively studied in eukaryotic cells (Ciechanover, 2014). Ub in its structure has seven lysine residues to be linked to other Ub-forming polyubiquitin chains. We can distinguish three types of ubiquitin attachment to a given protein depending on the number of ubiquitin molecules. Mono-ubiquitination: when a single Ub is linked to the substrate, resulting in transfer to endosomes, nuclear export, signal for DNA repair, and regulation of DNA transcription via chromatin remodeling. Multi-ubiquitination: when several single ubiquitin molecules are attached to several residues along the substrate, resulting in endo-cytosis. Lastly, polyubiquitination: the formation of polyubiquitin chains attached to the protein by the first ubiquitin (Pickart, 2004).

To add some more complexity to Ub-dependent protein trafficking, Ub in polyubiquitin chains can be linked to each other by one of seven lysines present in the Ub molecule, which determines the destination of protein with the attached Ub chain. For example, linking through 63 lysine (K63) leads proteins to aggre-gate with p62/SQSTM1 and degradation via autophagy (Wooten, 2008). Furthermore, the K63 chain also influences DNA repair and kinase activation (Acconcia, 2009). Finally, a chain consisting of at least four Ub monomers linked via K48 or K48/K11 is considered the most effective signal for degradation in proteasomes (Yau, 2017).

Polyubiquitination is controlled by three enzymes responsible for ubiquitin attachment. At the start, Ub is activated by forming an ATP-dependent thioester bond with the ubiquitin-activating enzyme (E1). Then activated Ub passes into the ubiquitin-conjugating enzyme (E2). Next, Ub linked with E2 is transferred to the target protein by ubiquitin-ligase enzyme (E3). Those actions repeat several times by bonding the next Ub to the previous one, creating a polyubiquitin chain (Hershko, 1998). In another pattern, when molecular chaperones fail to refold damaged proteins, another ubiquitin-ligase enzyme – cochaperone ubiquitin ligase carboxyl terminal of Hsp70/Hsp90 interacting protein (CHIP), performs ubiquitination and escorts proteins to the proteasome (Wojcik, 2002).

An ATP-dependent multi-catalytic protease (2,5 MDa) makes up the heart of the UPS – it is named 26S proteasome and is composed of 20S proteasome as tunnel-shaped core built up by 4 rings, each consisting of 7 globular particles: two peripheral alpha rings and two central beta rings; and two 19S regulatory subunits performing ubiquitin reception, deubiquitylating and unfolding amino acid chain, localized at the sides of the 20S proteasome. Three subunits in each beta ring determine the proteolytic activity of the 26S proteasome: beta1 with caspase-like activity, beta2 with trypsin-like activity, and beta5 with chymotrypsin-like activity (Manasanch, 2017). Oligopeptides leaving the 26S proteasome are further degraded by endo- and exopeptidases into amino acids (Saric, 2004).

UPS AND IMMUNITY

The UPS is involved in antigen turnover, especially endocellular antigen presentation, including viral antigen presentation. The IFN gamma facilitates both proteasome and antigen presentation for adequate clearance of viral infection (Seifert, 2010).

In somatic cells stimulated by interferon (INF)-gamma immunosubunits beta1i, beta2i, and beta5i are incorporated into proteasome structure in the places of beta1, beta2, and beta5 subunits. This newly rearranged structure is called an immunoproteasome (Basler, 2021). Changes in the structure of immunoproteasome result in altered proteolytic activity: Beta1-dependent caspase-like activity is reduced. Meanwhile, Beta5-dependent chymotrypsin-like activity is enormously increased. Resulting of these changes in proteolytic patterns, oligopeptides leaving proteasome mostly have hydrophobic amino acids on the c-terminal and are more efficiently presented by histocompatibility complex class I (MHC class I) (Groettrup, 2010). Through these changes, immunoproteasome increases peptide supply and provides more efficient antigen processing. Moreover, immunoproteasome helps to maintain proteostasis in the condition of increased oxidative stress promoted by INFs (Seifert, 2010). The altered proteasome is also involved in many processes inside the immunologic system or pathophysiology of neurodegenerative and inflammatory diseases (Basler, 2021).

Immunoproteasome manages with many different proteins, including DRiPS, which are particularly significant considering immune response (Seifert, 2010). Around 30% of newly synthesized proteins with an extremely short half-life (under 10 minutes) are called defective ribosomal products (DRiPs). They are defined as products of all mistakes in transcribing DNA information into protein (Qian, 2006; Anton, 2014). Even then, degradation via proteasome generates large quantities of antigens presented by MHC class I. Up to 70% of all antigens presented on cell surface come from DRiPs of cellular, viral, and neoplastic origin (Dolan, 2011). Moreover, they perform a crucial role in anti-viral response by rapidly presenting DRiPs-originated viral antigens enabling cytotoxic CD8+ T lymphocytes to recognize and kill infected cells before releasing new virions (Anton, 2014).

AUTOPHAGY AND UPS – ONE SYSTEM WITH TWO FACES

Cells under stress conditions, especially retrieval of nutrients, may activate autophagocytosis to obtain substrates for metabolism. While only specifically marked, small soluble proteins can be degraded by the proteasome, autophagy degrades protein polymers and all subcellular structures. Lysosomal hydrolases can digest different macromolecules: lipids, carbohydrates, proteins, intracellular aggregates, and even entire organelles inside endosomes fused with lysosomes. In the diversity of pathways leading to lysosome-mediated degradation, we can distinguish processes targeting monomeric molecules such as microautophagy and chaperone-mediated autophagy (Tekirdag, 2018). It is worth to add that macroautophagy is the essential type of autophagy in protein degradation. First, molecules are encircled by double-membraned structures called the autophagosome, then the material inside and the inner membrane are designated into degradation. Autophagy is considered the primary cellular response to acute nutrient depletion and is also involved in the degradation of protein aggregates, impaired organelles, and intracellular pathogens (Oh, 2018). Proteasomes can also be targeted by Ub and trafficked into autophagosomes in the process called proteophagy, probably as a mechanism of proteasome deactivation (Cohen-Kaplan, 2016). In amino acid deprivation, degradation via UPS is the first pathway to maintaining an adequate nutrient supply. Progressive amino acid deficiency blocks transcription and activates autophagy via the mTOR pathway (Suraweera, 2012).

Activation of ULK1 kinase in the pathway of the undefined enzymatic cascade induces the formation of the autophagosome and anchoring many members of the ATG8 protein family (a.o. LC3) to the phospholipids of growing membranes. Autophagic receptors (a.o. p62/SQSTM1), which direct particles to autophagosomes, have unique domains – LC3-interacting regions (Pohl, 2019). At the final stage of autophagy on SNARE-mediated fusion, autophagosomal and lysosomal membranes fuse. Then released lysosomal hydrolase in an acidic environment digests the inner autophagosomal membrane and phagosome contents into individual nutrients, which are transported to the cytosol (Yu, 2018). It is worth mentioning that p62 and other receptors equipped with Ubiquitin binding domains (UBDs) are situated at the crossroads of ubiquitination and autophagy (Rogov, 2014).

Interestingly, up to 50% of all selective autophagic particles are marked by Ub (Khaminets, 2016). p62 also acts in the UPS via its proteasome binding domains (PBD) by escorting ubiquitinylated molecules to the proteasome. According to recent studies, the shift between proteasomal and lysosomal degradation depends on the opposite polymerization and dimerization of p62 (Wurzer, 2015). Increased concentration of free Ub under prolonged stress or proteasome insufficiency probably block dimerization and promotes polymerization of p62, enhancing autophagy-dependent lysosomal degradation (Peng, 2017). Research

revealed complex correlations between UPS and autophagy, which preserve proteostasis. The pathways of these two systems overlap and complement each other. Nevertheless, in terms of insufficient degradation and formation of aggregates, a third cytoprotective mechanism is included – the formation of aggresomes.

AGGRESOME – SUBTLE RUBBISH BIN

Aggresomes are aggregates containing insoluble proteins, usually misfolded. They may also contain other molecules like cytoskeletal elements. Aggresomes are generally localized in the perinuclear region (Garcia-Mata, 1999; Johnston, 1998).

The massive juxtanuclear aggregates, later named aggresomes, had been described for the first time by Wójcik et al. in HeLa cells under proteasome inhibition. Researchers detected proteins designated for degradation, ubiquitin, and proteasome in these large insoluble aggregates and hypothesized lysosomal degradation of these structures under stress-free conditions (Wojcik, 1996). After the name "Aggresome" was proposed (Johnston, 1998), researchers revealed that the aggresomes are formed by the dynein-dependent retrograde transport of proteins and protein aggregates to the microtubule organizing center (MTOC) (Garcia-Mata, 1999). As a result, large unregular-shaped deposits are formed in the pericentriolar region near the Golgi apparatus. Interestingly, the accumulation of misfolded proteins around the centrosome does not interrupt ER-Golgi transport. Despite the total disorganization of MTOC, distal microtubules seem to perform their function unchanged (Garcia-Mata, 1999; Johnston, 1998). The formation of aggresomes interacts with the cytoskeleton in more ways. The intermediate filament (IF) cage established after the total reorganization of IFs surrounds the aggresome (Johnston, 1998). Aggresomes also contain damaged proteins, unbound ubiquitin, K48/K11, and K63 linked to the proteins, proteasome, chaperones Hsp40/Hsp70/Hsp90, ubiquitin ligases, histone deacetylase HDAC6, autophagy receptors (a.o. p62), tubulin and actin (Johnston, 2021). Aggresomes formation is crucial to cellular response when degradation via proteasome is overwhelmed by impaired proteins, both in proteasome inhibition, heat-shock response, and other stress conditions resulting in the accumulation of misfolded proteins. The cytoprotective character of aggresomes was confirmed by Nawrocki et al. in the condition of proteasome inhibition and combined disruption of aggresome formation (inhibition of HDAC6) survivability of cancerous cells was smaller than in cells affected only by proteasome inhibitor (Nawrocki, 2006; Mitsiades, 2004).

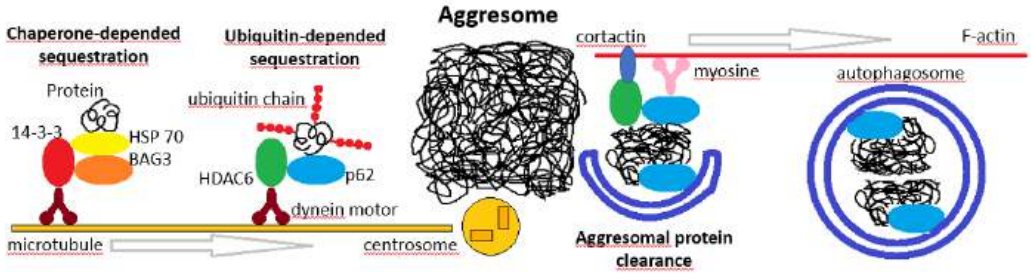

Figure 1. Formation and disruption of the aggresome. Two parallel processes can lead to aggresome formation. The first one: **Chaperone-dependent sequestration**: Involves BAG3 (co-chaperone) – that links misfolded protein tagged by HSP 70 to 14-3-3 protein. The latter 14-3-3 protein links the protein complex to dynein and traffic it to the centrosome. The second process: **Ubiquitin-dependent sequestration**: Involves poly-ubiquitin protein substrate that is linked to p62 protein and then is attached to microtubules via HDAC6 (Histone deacetylase). Both processes lead to formation of big protein aggregates in the cell center. The same two proteins, HDAC6 and p62 can promote: **Aggresomal protein clearance:** by association of the aggresome to cortactin (cortical actin binding protein) and motor it along actin filaments, depending on myosin, to the cell periphery where the aggresome can be cleared in autophagosome

HDAC6, despite its name, does not remodel chromatin but is involved in cytoplasmic pathways. This enzyme participates in heat-shock response as a co-chaperone regulating Hsp90 by deacetylation (Kovacs, 2005). More importantly, HDAC6 can deacetylate microtubules, thereby regulating dynein-dependent aggregates transport, or even function as a selective aggresome adaptor (Berdeja, 2021). Direct interactions between HDAC6 and p62 remain unknown, but their cooperation traffics ubiquitinated protein(K63)/p62 aggregates into aggresome rather than autophagy. Nevertheless, induced autophagy can also disrupt the formation of aggresomes. Phosphorylation of p62 blocks its LC3-interacting regions, thereby enabling autophagy and promoting protein sequestration into aggresomes, so inhibition of p62 phosphorylation

enhances autophagy and disrupts aggresomes formation (Zhang, 2022). On the other hand, p62, alongside HDAC6, promotes aggrephagy (autophagy of aggresome) by deacetylation of cortactin and myosin-dependent transport of misfolded protein aggregates from MOTC to peripheral cytosol, where aggregates are exposed for autophagosome (Yan, 2013).

Misfolded proteins may also be transferred to the aggresome in the Ub-independent pathway involving chaperones and BAG co-chaperone 3. BAG3 detects and directs the Protein-Hsp70 complex to the aggresome, using 14-3-3 protein. The whole transport complex is then linked to dynein, enabling retrograde transport along microtubules (Jia, 2014). This creates an additional chance to sequester into aggresome proteins which avoids ubiquitination. As well as p62-mediated transport, BAG3 can lead protein into selective autophagy (Gamerdinger, 2011).

Aggresome formation and disruption mechanisms remain primarily unexplored. Nevertheless, described interactions show how efficiently aggresome formation complements proteasomal degradation. Future research is needed to reveal the nature of these mechanisms, considering that aggresomes are involved in the pathogenesis of many diseases.

AGGRESOMES – AT THE CENTER OF CONFORMATIONAL DISEASES

Disturbed proteostasis underlies the pathogenesis of many severe and lethal diseases. Accumulation of misfolded proteins and their aggregation into insoluble deposits may impair cell functions and promote cell death. The presence of dysfunctional proteins promotes their aggregation into stable beta-pleated sheets of amyloid fibrils, plaques, or tangles, both endoplasmic and extracellular (Chiti, 2017; Babu, 2011). Amyloid formations present their characteristic morphological structure, even though their monomers originate from proteins with various native conformations. Aggregates lay at the basis of many chronic diseases, including multiple types of amyloidosis – a broad group of systemic and localized diseases with similar morphological appearances centered around amyloid deposits (Hazenberg, 2013). For example, deposits of the protein named islet amyloid polypeptide (IAPP) are connected to type 2 diabetes progression by damaging the islets of Langerhans (Shahnawaz, 2017). Misfolded proteins can also aggre-gate into soluble oligomers, which seem more cytotoxic than amyloid structures (Walsh, 2004; Caughey, 2003). This argument may support the hypothesis that aggregation is a cytoprotective mechanism that becomes impaired in conformational diseases.

Among other pathologies related to aggregate accumulation, neurodegenerative diseases have the most significant social impact. Besides genetic predispositions and environmental conditions, it was revealed that aging impact proteostasis by the expansion of misfolded proteins and limited proteolysis (Olzmann, 2008). This molecular mechanism justifies calling these diseases age-related. Due to their sophisticated struc-ture, neurons are especially vulnerable to the destructive effects of pathogenic inclusions. The aggregate formation is the crucial pathogenesis mechanism of Alzheimer's disease – beta-amyloid deposits and tau neurofibrils, Parkinson's disease – Lewy bodies, Huntington's disease – PolyQ bodies, and many other neurodegenerative disorders (Dugger, 2017).

Interestingly, these inclusions are biochemically and morphologically similar to aggresomes. Supposably their formation depends on the same pathways. Furthermore, it was shown that all-important particles of the aggresome: proteasome, chaperones, gamma-tubulin, HDAC6, and p62 are incorporated into the structure of Lewy bodies inside glial cells (Chiba, 2012). Parallelly, researchers revealed that aggresome adaptors – HDAC6, p62, and 14-3-3, play crucial roles in neurodegenerative disease pathogenesis, and more importantly, their pathways may have therapeutic significance (Jia, 2014; Ma, 2019).

PROTEASOME INHIBITORS – THE CLINICAL IMPLICATION OF PROTEOSTASIS

Progressing carcinogenesis is strictly correlated with the accumulation of mutations, genetic instability, and loss of control mechanisms. Rapid growth and short cell cycle of cancer cells require an increased flow of newly synthesized proteins (Hanahan, 2011). However, it makes neoplastic cells more vulnerable to disturbances in the UPS pathway, which is overcome by the supply of proteins. The primary mechanism of action of proteasome inhibitors (PI) in cancer therapy is the blockage of the UPS cytoprotective pathway. PIs increase the concentration of damaged proteins and induce unfolded protein response (UPR), which results in cell cycle arrest and apoptosis of neoplastic cells (Nunes, 2017). This pathway also explains why cancer cells that produce large quantities of protein are more vulnerable to PI-mediated proteostasis

disruption than healthy cells (Mlynarczuk-Bialy, 2014; Mlynarczuk-Bialy, 2006). This difference is especially useful in treating malignancies originating from plasma cells that produce large amounts of immunoglobins.

PIs also promote cell death affecting many other pathways. The best-described cytotoxicity mechanism of PIs, besides UPR inducement, is inhibiting the pro-survival anti-apoptotic NF-κB pathway, which is also involved in angiogenesis, metastasis, and invasion of cancer cells (Nunes, 2006). After receiving an activating stimuli, an inhibitor of this pathway – IκBα is degraded via UPS, which releases transcription factor NF-κB and transfers it into the nucleus (Palombella, 1994). Proteasome inhibition prevents the release of NF-κB, therefore, arresting the NF-κB pathway, effectively turning off pro-survival stimulation. In other patterns, PIs may induce apoptosis directly via activation c-Jun NH2-terminal kinase (JNK) pathway resulting in activation of caspase 8 and caspase 3 (Dai, 2003), overexpression of p53 regulating protein (An, 2006), or accumulation of pro-apoptotic Bim, Bik, and Bid proteins (Nunes, 2017; Breitschopf, 2000).

Proteasome inhibitors were initially developed as agents that prevent cancer-related cachexia by a decrease of UPS-mediated degradation and protein turnover. Subsequently, preclinical research revealed their pro-apoptotic activity *in vitro* and *in vivo* murine cancer models, which suggested potential chemotherapeutic utility (Manasanch, 2017). Two decades of development of PIs resulted in clinical validation and regulatory approval in the usage of three compounds for the treatment of multiple myeloma and mantle-cell lymphoma: bortezomib (PS-341, VELCADE) – a first-generation agent, carfilzomib (KYPROLIS) and ixazomib (NINLARO) – second-generation agents (Richardson, 2003; Alsina, 102; Philippe Moreau, 2015). Each presents inhibitory activity towards beta5 and beta5i subunits (in higher concentrations, these compounds inhibit beta1 and beta2 subunits as well), inhibiting both constitutive 26S proteasome and immunoproteasome (Wang, 2021). Interestingly, *in vitro* usage of selective constitutive proteasome or immunoproteasome inhibitors does not induce apoptosis in myeloma cells line (Eleftheriadis, 2017), which may confirm the involvement of immunoproteasome in the maintenance of proteostasis in hemato-poietic cells lines.

Table 1. Properties of proteasome inhibitors approved and in clinical trials

Proteasome inhibitor	Class	Targets	Administration	Approved in
Bortezomib	Boronate/reversible	Constitutive and immunoproteasome	IV and SC	multiple myeloma, mantle-cell lymphoma
Carfilzomib	Epoxyketone/irreversible	Constitutive and immunoproteasome	IV	multiple myeloma
Ixazomib	Boronate/reversible	Constitutive and immunoproteasome	IV and Oral	multiple myeloma
Marizomib	cyclic beta-lactone gamma-lactam/ irreversible	Constitutive and immunoproteasome	IV	-----
KZR-616	Epoxyketone/irreversible	immunoproteasome	SC	-----

PROTEASOME-TARGETED THERAPIES

Due to the impressive results of bortezomib treatment of relapsed/refractory multiple myeloma in phase II clinical trial (Richardson, 2003), FDA approved bortezomib on the expedited procedure as salvage therapy in 2003. Next, full regulatory approval of the first PI in multiple myeloma appeared in 2005, after an extended phase III trial (Richardson, 2005). Soon after, bortezomib was approved for treating mantle cell lymphoma (Fisher, 2006). Bortezomib consists of a dipeptide backbone connected to a boronate group, which interacts with catalytic threonine residue in proteasome subunits, reversibly inhibiting them (Schrader, 2016). Bortezomib is administrated as a mannitol ester by intravenous (IV) or subcutaneous (SC) route. Both administration routes present the same anti-tumor activity. Then, the drug quickly leaves the vascular compartment and, in therapeutic concentration, may result in maximum inhibition of proteasome up to 74% (Orlowski, 2002). Bortezomib is metabolized intrahepatic through oxidative deboronation performed by CYP450 enzymes and is excreted in bile and urine. Combination treatment with other chemotherapeutics has been referred to as more effective than bortezomib monotherapy. A widely used therapy option in treating multiple myeloma is a combination of bortezomib with dexa-methasone and lenalidomide- leading to prolonged remissions and improving overall survival compared to

dexamethasone-lenalidomide treatment (Durie, 2020). Bortezomib can also be combined with doxorubicin and dexamethasone as maintenance therapy in newly diagnosed multiple myeloma (Sonneveld, 2012). A combination of bortezomib and dexamethasone with daratumumab – an anti-CD38 monoclonal antibody, obtained stunning results in treating multiple myeloma (Palumbo, 2016). However, in this paper, we would like to distinguish the approved by FDA therapy for patients with multiple myeloma who have received at least two prior lines of treatment, a combination of dexamethasone, bortezomib, and panobinostat – HDAC inhibitor, which disturbs aggresome formation (San-Miguel, 2016; Dimopoulos, 2022). A severe problem for bortezomib-treated patients and a dose-limiting factor is the development of peripheral neuropathy. Even 80% of patients treated with bortezomib develop this condition (Richardson, 2010). Thrombocytopenia, nausea, diarrhea, fatigue, and neutropenia are the other side effects. Combination treatment with bortezomib used in another type of lymphoma, Waldenstrom's macroglobulinemia, is effective and is used in clinical practice (Ghobrial, 2010). Interestingly, bortezomib's potential to induce proteotoxicity helps treat light-chain amyloidosis, a disease caused by the deposition of light chains secreted by malignancy plasma cells into beta-pleated sheets of amyloid structures (Sanchorawala, 2015). Bortezomib did not show results in treating other hematological malignancies and solid tumors. The possible cause of these failures is relatively high toxicity, hence administration below the effective therapeutic dose. The solution to these problems may be to use second-generation PIs.

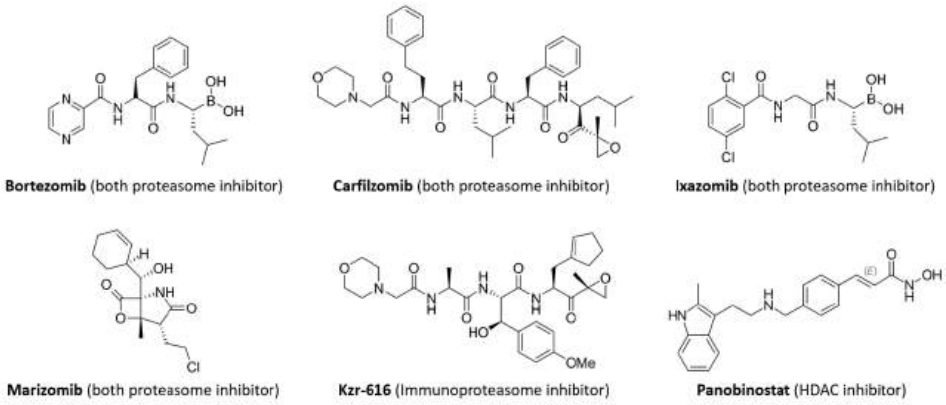

Figure 2. Drugs affecting proteostasis – proteasome inhibitors and Panobinostat

Carfilzomib consists of a tetrapeptide backbone and epoxyketone group as a warhead, which irreversible and specifically binds with threonine residue in catalytic proteasome subunits, unlike bortezomib. The drug is administered via the IV route, then rapidly leaves the vascular compartment. The T1/2 value for carfilzomib is under 30 min, which suggests extrahepatic metabolism. First, elimination includes epoxide hydrolyzation performed by microsomal epoxide hydrolases and peptidase cleavage. Next, carfilzomib is excreted in bile and urine (Alsina, 2012; Perel, 2016). Second-generation PI Carfilzomib was approved by FDA in 2012 as monotherapy, based on results of clinical application (Alsina, 2012), but nowadays is more often used in combination therapies. As bortezomib, carfilzomib combined with lenalidomide and dexamethasone is used to treat patients with multiple myeloma (Lendvai, 2014), and usage with panobinostat is under evaluation (Berdeja, 2015; Berdeja, 2021). The second widely used combination therapy that includes carfilzomib is treatment with melphalan and prednisone – drugs also used with bortezomib. Comparative phase III clinical trials do not indicate significant differences in anti-tumor activity in patients with multiple myeloma between these two combination therapies (Facon, 2019). Differences in the clinical application are mainly based on different toxicity profiles of bortezomib and carfilzomib, the latter not causing peripheral neuropathy but a dose-limiting deep vein thrombosis and febrile neutropenia. Other side effects are hypo and hypertension, fever, anemia, fatigue, cardiac failure, and shortness of breath (Manasanch, 2017).

Ixazomib, bortezomib analog bioavailable in oral administration, is the third PI approved by FDA in 2015 in combination therapy with dexamethasone and lenalidomide in patients with relapsed refractory multiple myeloma (Philippe Moreau, 2015). The mechanism of reversible proteasome inhibition resembles bortezomib action (Schrader, 2016). Ixazomib can be administrated in both IV and oral route as Ixazomib citrate, which rapidly converts into active form after exposure to plasma (Kupperman, 2010). The drug

absorbs quickly after oral administration (bioavailability of 58%) and is slowly removed from plasma with t1/2 of 9,5 days (Assouline, 2014). Metabolism of ixazomib is also like bortezomib once. In terms of toxicity, patients treated with ixazomib present peripheral neuropathy, thrombocytopenia, and gastro-intestinal disorders (Chen, 2021).

Marizomib (salinosporamide A) is another inhibitor of constitutive proteasome and immunoproteasome, but in contrast to others, PIs irreversibly inhibit all three catalytic subunits of the proteasome. It also belongs to another chemical group – cyclic beta-lactone gamma-lactam and is produced naturally by the marine bacteria *Salinispora Tropica* (Potts, 2011). The drug does not present significant toxicity. Side effects are limited to nausea, diarrhea, and fatigue. Marizomib is currently under evaluation for treating multiple myeloma and glioblastoma (Kisselev, 2021). In addition, due to its more hydrophobic structure, marizomib, unlike other PIs, can cross the blood-brain barrier, making it a potential option for treating brain tumors (Di, 2016).

Research on tool PI ONX 0914 found that selective immunoproteasome inhibition limits inflammatory activity without the cytotoxic effect of dual proteasome inhibition in preclinical models (Muchamuel, 2009). Soon after, researchers discovered the ability of the KZR-616 compound to inhibit immunoproteasomes selectively with good pharmaceutical properties. The KZR-616 is a tri-peptide analog of carfilzomib with an epoxyketone warhead, with binds irreversibly to beta5i (inhibits nearly 100% of chymotrypsin-like activity) and beta1i (inhibits almost 40% of caspase-like activity) (Johnson, 2018). The drug is administrated SC, with a bioavailability of nearly 100%. Like carfilzomib, absorption, and clearance of KZR-616 are rapid, but human metabolism is performed only by microsomal epoxide hydrolases (Fang, 2021). KZR-616 is being evaluated in patients with autoimmune disorders – lupus nephritis, polymyositis, and dermatomyositis in phase II clinical trials (Johnson, 2018). Patients also received standard immuno-suppressing drugs and corticosteroids without signs of drug-drug interactions. Inhibition of immunopro-teasome results in reduced cytokine expression, decreased Th1 and Th17 cell differentiation, reduced amounts of class-switched plasma cells, and a significant decrease in autoimmune antibody concentration (Basler, 2021; Kirk, 2021). Also, in the murine polymyositis model, KZR-616 significantly improves muscle function (Del Rio Oliva, 2022). No serious adverse events were reported in patients treated with KZR-616, in contrast to dual PIs. Side effects are limited to erythema in the place of injection (Kirk, 2021).

HDAC6 AND AGGRESOME FORMATION PATHWAY – WAY TO OVERCOME RESISTANCE TO PIS

Conversely, resistance to PIs in tumor cells limits their therapeutic applications (Merin, 2014). Mecha-nisms of resistance, despite the knowledge of some pathways, remain mostly undiscovered. Nevertheless, each newly discovered pathway brings us closer to understanding the effects of PIs treatment on cancer cells, which may help develop novel therapies. As one of the discovered mechanisms, some neoplastic cells resistant to PIs present a mutation in gene-coding beta5 catalytic subunits, preventing drugs from binding (Lu, 2009). Also, overexpression of proteasome subunits and chaperones may support proteostasis under stress induced by proteasome inhibition (Fuchs, 2008). An overload with toxic misfolded proteins during the insufficiency of UPS has to be either degraded by other enzymes, including lysosomal hydrolases in autophagy, or be sequestrated into aggresomes. Unsurprisingly, aggresomes were initially observed in cancer cell lines under proteasome inhibition (Wojcik, 1996). The cytoprotective character of aggresomes has a significant impact on tumor cells' survivability under proteotoxic stress. Thus, disruption of aggresome formation results in increased vulnerability to misfolded protein stress. The selective aggres-ome adaptor – HDAC6 that traffics misfolded proteins and smaller aggregates into aggresomes may be inhibited, which results in foreclosing of the aggresomes formation pathway from the proteostasis network (Rodriguez-Gonzalez, 2008). The importance of aggresome formation in cells resistant to PIs was revealed in many preclinical studies, and HDAC6 inhibition was proven to be an effective way of reducing the survivability of cancer cells in both *in vitro* and *in vivo* models (Nawrocki, 2006; Mitsiades, 2004). Moreover, HDAC6 inhibition proved to be effective in clinical applications.

Panobinostat (FARYDAC) – the first HDACs inhibitor that affects all enzymes of this class, did not present a therapeutic effect in monotherapy (Wolf, 2012). However, in phase III clinical trials combination treatment of bortezomib and dexamethasone proved to be effective in patients with refractory/relapsed multiple myeloma (San-Miguel, 2016), which resulted in FDA approval in 2015 of this therapy in patients who have been treated with at least two lines of treatment including bortezomib and an immunomodula-ting drug. Panobinostat inhibits all four classes of HDACs, mostly inactivating HDAC1-3 and HDAC6 (Berdeja,

2021). Inhibition of HDAC1-3 leads to epigenetic changes, transcription of suppressor genes, and enhanced synthesis of proteins. On the other hand, HDAC6 inhibition results in the inhibition of HSP90 and disruption of aggresome formation (Kovacs, 2005; Berdeja, 2021). In contrast to other HDACs inhibitors approved in clinical usage: vorinostat, belinostat, and romidepsin (which seem not to disturb proteostasis), panobinostat combination therapy mainly relies on dysregulation of protein metabolism. Panobinostat combination therapies with second-generation PIs – carfilzomib (Berdeja, 2015; Berdeja, 2021) and ixazomib plus dexamethasone (NCT02057640) are currently under evaluation. Panobinostat is a hydroxamic acid-based compound administered IV or orally (bioavailability of 21%), then rapidly absorbed. The T1/2 value of panobinostat is 31 hours (Srinivas, 2017). The drug is metabo-lized mainly by CYP450 enzymes in the liver and is excreted in bile and urine. The toxicity profile of panobinostat does not include serious side effects. The most common side effects include thrombocyto-penia, neutropenia, and gastrointestinal disorders (Berdeja, 2021).

DISCUSSION

Understanding the cellular mechanism and their pathology in cancer cells is crucial for developing new therapies. This also applies to the network of proteostasis and related therapies. The discovery of the proteasome and the UPS pathway enables the development of PIs and their application to the treatment of hematological malignancies (Richardson, 2007; Alsina, 2012; Philippe Moreau, 2015). Furthermore, there is still a place for research on the new PIs. Newly synthesized molecules may be more cytotoxic to cancer cells and have a better toxicity profile. As a result, they may be applied to the treatment of a broad spectrum of malignancies. Currently approved PIs can't be used in treating solid tumors, but the development of newly synthesized PIs may overcome this. Moreover, in the era of a personalized medicine board range of possible drugs, may help to suit the best therapeutic option for the patient.

Panobinostat shows that proteostasis mechanisms beyond the proteasome are also possible targets in cancer treatment (San-Miguel, 2016). Disturbance in aggresome formation by inhibition of HDAC6 acts parallelly with bortezomib. This approach makes the aggresome formation pathway a vital target in the therapy of relapsed refractory multiple myeloma. Furthermore, other drugs affecting the proteostasis network are under evaluation. Pevonedistat – an inhibitor of the ubiquitin-activating enzyme (E1) NEDD-8, and RG7112 – an inhibitor of the ubiquitin-ligase enzyme (E3) MDM2, have shown activity against acute myeloid leukemia (Manasanch, 2017). More research is needed to evaluate these drugs in a clinical approach.

The research on proteostasis revealed the main patterns in cellular protein turnover and degradation pathways. Nevertheless, details in these pathways remain unclear. May the discovery of detailed interaction in the proteostasis network will enable the development of new drugs in therapeutic options in cancer therapy.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Acconcia F., Sigismund S., Polo S. **Ubiquitin in trafficking: the network at work**. Exp Cell Res. 2009; 315:1610-1618.

Almanza A., Carlesso A., Chintha C., Creedican S., Doultsinos D., Leuzzi B., et al. **Endoplasmic reticulum stress signalling - from basic mechanisms to clinical applications**. FEBS J. 2019; 286: 241-278.

Alsina M., Trudel S., Furman R.R., Rosen P.J., O'Connor O.A., Comenzo R.L., et al. **A phase I single-agent study of twice-weekly consecutive-day dosing of the proteasome inhibitor carfilzomib in patients with relapsed or refractory multiple myeloma or lymphoma**. Clin Cancer Res. 2012; 18: 4830-4840.

An W.G., Hwang S.G., Trepel J.B., Blagosklonny M.V. **Protease inhibitor-induced apoptosis: accumulation of wt p53, p21WAF1/CIP1, and induction of apoptosis are independent markers of proteasome inhibition**. Leukemia. 2000; 14: 1276-1283.

Anton L.C., Yewdell J.W. **Translating DRiPs: MHC class I immunosurveillance of pathogens and tumors**. J Leukoc Biol. 2014; 95: 551-562.

Assouline S.E., Chang J., Cheson B.D., Rifkin R., Hamburg S., Reyes R., et al. **Phase 1 dose-escalation study of IV ixazomib, an investigational proteasome inhibitor, in patients with relapsed/refractory lymphoma**. Blood Cancer J. 2014; 4: e251.

Babu M.M., van der Lee R., de Groot N.S., Gsponer J. **Intrinsically disordered proteins: regulation and disease**. Curr Opin Struct Biol. 2011; 21: 432-440.

Basler M., Groettrup M. **On the Role of the Immunoproteasome in Protein Homeostasis**. Cells. 2021; 10.

Berdeja J.G., Gregory T.K., Faber E.A., Hart L.L., Mace J.R., Arrowsmith E.R., et al. **A phase I/II study of the combination of panobinostat and carfilzomib in patients with relapsed or relapsed/refractory multiple myeloma: Final analysis of second dose-expansion cohort**. Am J Hematol. 2021; 96: 428-435.

Berdeja J.G., Hart L.L., Mace J.R., Arrowsmith E.R., Essell J.H., Owera R.S., et al. **Phase I/II study of the combination of panobinostat and carfilzomib in patients with relapsed/refractory multiple myeloma**. Haematologica. 2015; 100: 670-676.

Berdeja J.G., Laubach J.P., Richter J., Stricker S., Spencer A., Richardson P.G., et al. **Panobinostat From Bench to Bedside: Rethinking the Treatment Paradigm for Multiple Myeloma**. Clin Lymphoma Myeloma Leuk. 2021; 21: 752-765.

Bonifacino J.S., Suzuki C.K., Lippincott-Schwartz J., Weissman A.M., Klausner R.D. **Pre-Golgi degradation of newly synthesized T-cell antigen receptor chains: intrinsic sensitivity and the role of subunit assembly**. J Cell Biol. 1989; 109: 73-83.

Breitschopf K., Zeiher A.M., Dimmeler S. **Ubiquitin-mediated degradation of the proapoptotic active form of bid. A functional consequence on apoptosis induction**. J Biol Chem. 2000; 275: 21648-21652.

Caughey B., Lansbury P.T. **Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders**. Annu Rev Neurosci. 2003; 26: 267-298.

Chen H., Wang Y., Shao C., Sun C., Zhen C. **Efficacy and safety of ixazomib maintenance therapy for patients with multiple myeloma: a meta-analysis**. Hematology. 2021; 26: 1031-1039.

Chiba Y., Takei S., Kawamura N., Kawaguchi Y., Sasaki K., Hasegawa-Ishii S., et al. **Immunohistochemical localization of aggresomal proteins in glial cytoplasmic inclusions in multiple system atrophy**. Neuropathol Appl Neurobiol. 2012; 38: 559-571.

Chiti F., Dobson C.M. **Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade**. Annu Rev Biochem. 2017; 86: 27-68.

Ciechanover A., Stanhill A. **The complexity of recognition of ubiquitinated substrates by the 26S proteasome**. Biochim Biophys Acta.2014; 1843: 86-96.

Cohen-Kaplan V., Livneh I., Avni N., Fabre B., Ziv T., Kwon Y.T., et al. **p62- and ubiquitin-dependent stress-induced autophagy of the mammalian 26S proteasome**. Proc Natl Acad Sci U S A. 2016; 113: E7490-E7499.

Dai Y., Rahmani M., Grant S. **Proteasome inhibitors potentiate leukemic cell apoptosis induced by the cyclin-dependent kinase inhibitor flavopiridol through a SAPK/JNK- and NF-kappaB-dependent process**. Oncogene 2003; 22: 7108-7122.

Del Rio Oliva M., Kirk C.J., Groettrup M., Basler M. **Effective therapy of polymyositis in mice via selective inhibition of the immunoproteasome**. Eur J Immunol. 2022; 52: 1510-1522.

Di K., Lloyd G.K., Abraham V., MacLaren A., Burrows F.J., Desjardins A., et al. **Marizomib activity as a single agent in malignant gliomas: ability to cross the blood-brain barrier**. Neuro Oncol. 2016; 18: 840-848.

Dimopoulos M.A., Richardson P., Lonial S. **Treatment Options for Patients With Heavily Pretreated Relapsed and Refractory Multiple Myeloma**. Clin Lymphoma Myeloma Leuk. 2022; 22: 460-473.

Dolan B.P., Bennink J.R., Yewdell J.W. **Translating DRiPs: progress in understanding viral and cellular sources of MHC class I peptide ligands**. Cell Mol Life Sci. 2011; 68: 1481-1489.

Dugger B.N., Dickson D.W. **Pathology of Neurodegenerative Diseases**. Cold Spring Harb Perspect Biol. 2017; 9.

Durie B.G.M., Hoering A., Sexton R., Abidi M.H., Epstein J., Rajkumar S.V., et al. **Longer term follow-up of the randomized phase III trial SWOG S0777: bortezomib, lenalidomide and dexamethasone vs. lenalidomide and dexamethasone in patients (Pts) with previously untreated multiple myeloma without an intent for immediate autologous stem cell transplant (ASCT)**. Blood Cancer J. 2020; 10: 53.

Eleftheriadis T., Pissas G., Antoniadi G., Liakopoulos V., Stefanidis I. **A comparative analysis between proteasome and immunoproteasome inhibition in cellular and humoral alloimmunity**. Int Immunopharmacol. 2017; 50: 48-54.

Facon T., Lee J.H., Moreau P., Niesvizky R., Dimopoulos M., Hajek R., et al. **Carfilzomib or bortezomib with melphalan-prednisone for transplant-ineligible patients with newly diagnosed multiple myeloma**. Blood. 2019; 133: 1953-1963.

Fang Y., Johnson H., Anderl J.L., Muchamuel T., McMinn D., Morisseau C., et al. **Role of Epoxide Hydrolases and Cytochrome P450s on Metabolism of KZR-616, a First-in-Class Selective Inhibitor of the Immunoproteasome**. Drug Metab Dispos. 2021; 49: 810-821.

Fisher R.I., Bernstein S.H., Kahl B.S., Djulbegovic B., Robertson M.J., de Vos S., et al. **Multicenter phase II study of bortezomib in patients with relapsed or refractory mantle cell lymphoma**. J Clin Oncol. 2006; 24: 4867-4874.

Fuchs D., Berges C., Opelz G., Daniel V., Naujokat C. **Increased expression and altered subunit composition of proteasomes induced by continuous proteasome inhibition establish apoptosis resistance and hyperproliferation of Burkitt lymphoma cells**. J Cell Biochem. 2008; 103: 270-283.

Gamerdinger M., Kaya A.M., Wolfrum U., Clement A.M., **Behl C. BAG3 mediates chaperone-based aggresome-targeting and selective autophagy of misfolded proteins**. EMBO Rep. 2011; 12: 149-156.

Garcia-Mata R., Bebok Z., Sorscher E.J., Sztul E.S. **Characterization and dynamics of aggresome formation by a cytosolic GFP-chimera**. J Cell Biol. 1999; 146: 1239-1254.

Ghobrial I.M., Xie W., Padmanabhan S., Badros A., Rourke M., Leduc R., et al. **Phase II trial of weekly bortezomib in combination with rituximab in untreated patients with Waldenstrom Macroglobulinemia**. Am J Hematol. 2010; 85: 670-674.

Glickman M.H., Ciechanover A. **The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction**. Physiol Rev. 2002; 82: 373-428.

Groettrup M., Kirk C.J., Basler M. **Proteasomes in immune cells: more than peptide producers?** Nat Rev Immunol. 2010; 10: 73-78.

Haglund K., Dikic I. **Ubiquitylation and cell signaling**. EMBO J. 2005; 24: 3353-3359.

Hanahan D., Weinberg R.A. **Hallmarks of cancer: the next generation**. Cell. 2011; 144: 646-674.

Harper J.W., Bennett E.J. **Proteome complexity and the forces that drive proteome imbalance**. Nature. 2016; 537: 328-338.

Hazenberg B.P. **Amyloidosis: a clinical overview**. Rheum Dis Clin North Am. 2013; 39: 323-345.

Hershko A., Ciechanover A. **The ubiquitin system**. Annu Rev Biochem. 1998; 67: 425-479.

Jayaraj G.G., Hipp M.S., Hartl F.U. **Functional Modules of the Proteostasis Network**. Cold Spring Harb Perspect Biol. 2020; 12.

Jia B., Wu Y., Zhou Y. **14-3-3 and aggresome formation: implications in neurodegenerative diseases**. Prion. 2014; 8.

Johnson H.W.B., Lowe E., Anderl J.L., Fan A., Muchamuel T., Bowers S., et al. **Required Immunoproteasome Subunit Inhibition Profile for Anti-Inflammatory Efficacy and Clinical Candidate KZR-616 ((2 S,3 R)- N-((S)-3-(Cyclopent-1-en-1-yl)-1-((R)-2-methyloxiran-2-yl)-1-oxopropan-2-yl)-3-hydroxy-3-(4-methoxyphenyl)-2-((S)-2-(2-morpholinoacetamido)propanamido)propenamide)**. J Med Chem. 2018; 61: 11127-11143.

Johnston H.E., Samant R.S. **Alternative systems for misfolded protein clearance: life beyond the proteasome**. FEBS J. 2021; 288: 4464-4487.

Johnston J.A., Ward C.L., Kopito R.R. **Aggresomes: a cellular response to misfolded proteins**. J Cell Biol. 1998; 143: 1883-1898.

Khaminets A., Behl C., Dikic I. **Ubiquitin-Dependent And Independent Signals In Selective Autophagy**. Trends Cell Biol. 2016; 26: 6-16.

Kirk C.J., Muchamuel T., Wang J., Fan R.A. **Discovery and Early Clinical Development of Selective Immunoproteasome Inhibitors**. Cells. 2021; 11.

Kisselev A.F., **Site-Specific Proteasome Inhibitors**. Biomolecules. 2021; 12.

Kovacs J.J., Murphy P.J., Gaillard S., Zhao X., Wu J.T., Nicchitta C.V., et al. **HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor**. Mol Cell. 2005; 18: 601-607.

Kupperman E., Lee E.C., Cao Y., Bannerman B., Fitzgerald M., Berger A., et al. **Evaluation of the proteasome inhibitor MLN9708 in preclinical models of human cancer**. Cancer Res. 2010; 70: 1970-1980.

Lendvai N., Hilden P., Devlin S., Landau H., Hassoun H., Lesokhin A.M., et al. **A phase 2 single-center study of carfilzomib 56 mg/m2 with or without low-dose dexamethasone in relapsed multiple myeloma**. Blood. 2014; 124: 899-906.

Lu S., Yang J., Chen Z., Gong S., Zhou H., Xu X., et al. **Different mutants of PSMB5 confer varying bortezomib resistance in T lymphoblastic lymphoma/leukemia cells derived from the Jurkat cell line**. Exp Hematol. 2009; 37: 831-837.

Ma S., Attarwala I.Y., Xie X.Q. SQSTM1/p62: **A Potential Target for Neurodegenerative Disease**. ACS Chem Neurosci. 2019; 10: 2094-2114.

Manasanch E.E., Orlowski R.Z. **Proteasome inhibitors in cancer therapy**. Nat Rev Clin Oncol. 2017; 14: 417-433.

Merin N.M., Kelly K.R. **Clinical use of proteasome inhibitors in the treatment of multiple myeloma**. Pharmaceuticals (Basel). 2014; 8: 1-20.

Mitsiades C.S., Mitsiades N.S., McMullan C.J., Poulaki V., Shringarpure R., Hideshima T., Akiyama M., et al. **Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications**. Proc Natl Acad Sci U S A. 2004; 101: 540-545.

Mlynarczuk-Bialy I., Doeppner T.R., Golab J., Nowis D., Wilczynski G.M., Parobczak K., et al. **Biodistribution and Efficacy Studies of the Proteasome Inhibitor BSc2118 in a Mouse Melanoma Model**. Transl Oncol. 2014; 7: 570-579.

Mlynarczuk-Bialy I., Roeckmann H., Kuckelkorn U., Schmidt B., Umbreen S., Golab J., et al. **Combined effect of proteasome and calpain inhibition on cisplatin-resistant human melanoma cells**. Cancer Res. 2006; 66: 7598-7605.

Moreau P., Masszi T., Grzasko N., Bahlis N.J., Hansson M., Pour L., et al. **Ixazomib, an Investigational Oral Proteasome Inhibitor (PI), in Combination with Lenalidomide and Dexamethasone (IRd), Significantly Extends Progression-Free Survival (PFS) for Patients (Pts) with Relapsed and/or Refractory Multiple Myeloma (RRMM): The Phase 3 Tourmaline-MM1 Study (NCT01564537)**. Blood. 2015; 126 (123): 727.

Muchamuel T., Basler M., Aujay M.A., Suzuki E., Kalim K.W., Lauer C., et al. **A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis**. Nat Med. 2009; 15: 781-787.

Nawrocki S.T., Carew J.S., Pino M.S., Highshaw R.A., Andtbacka R.H., Dunner K., et al. **Aggresome disruption: a novel strategy to enhance bortezomib-induced apoptosis in pancreatic cancer cells**. Cancer Res. 2006; 66: 3773-3781.

Nunes A.T., Annunziata C.M. **Proteasome inhibitors: structure and function**. Semin Oncol. 2017; 44: 377-380.

Oh E., Akopian D., Rape M. **Principles of Ubiquitin-Dependent Signaling**. Annu Rev Cell Dev Biol. 2018; 34: 137-162.

Olzmann J.A., Li L., Chin L.S. **Aggresome formation and neurodegenerative diseases: therapeutic implications**. Curr Med Chem. 2008; 15: 47-60.

Orlowski R.Z., Stinchcombe T.E., Mitchell B.S., Shea T.C., Baldwin A.S., Stahl S., et al. **Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies**. J Clin Oncol. 2002; 20: 4420-4427.

Palombella R.O., Goldberg A.L., Maniatis T., Palombella V.J., Rando O.J., Goldberg A.L., et al. **Cell**. 1994; 78(75): 773-785.

Palumbo A., Chanan-Khan A., Weisel K., Nooka A.K., Masszi T., Beksac M., et al. **Daratumumab, Bortezomib, and Dexamethasone for Multiple Myeloma**. N Engl J Med. 2016; 375: 754-766.

Peng H., Yang J., Li G., You Q., Han W., Li T., et al. **Ubiquitylation of p62/sequestosome1 activates its autophagy receptor function and controls selective autophagy upon ubiquitin stress**. Cell Res. 2017; 27: 657-674.

Perel G., Thomas C.M. Carfilzomib (Kyprolis): **A Novel Proteasome Inhibitor for Relapsed And/or Refractory Multiple Myeloma**. P T. 2016: 41(45): 303-307.

Pickart C.M., Fushman D. **Polyubiquitin chains: polymeric protein signals**. Curr Opin Chem Biol. 2004; 8: 610-616.

Pohl C., Dikic I. **Cellular quality control by the ubiquitin-proteasome system and autophagy**. Science. 2019; 366: 818-822.

Potts B.C., Albitar M.X., Anderson K.C., Baritaki S., Berkers C., Bonavida B., et al. **Marizomib, a proteasome inhibitor for all seasons: preclinical profile and a framework for clinical trials**. Curr Cancer Drug Targets. 2011; 11: 254-284.

Qian S.B., Princiotta M.F., Bennink J.R., Yewdell J.W. **Characterization of rapidly degraded polypeptides in mammalian cells reveals a novel layer of nascent protein quality control**. J Biol Chem. 2006; 281: 392-400.

Richardson P.G., Barlogie B., Berenson J., Singhal S., Jagannath S., Irwin D., et al. **A phase 2 study of bortezomib in relapsed, refractory myeloma**. N Engl J Med. 2003; 348: 2609-2617.

Richardson P.G., Sonneveld P., Schuster M.W., Irwin D., Stadtmauer E.A., Facon T., et al. **Assessment of Proteasome Inhibition for Extending Remissions, Bortezomib or high-dose dexamethasone for relapsed multiple myeloma**. N Engl J Med. 2005; 352: 2487-2498.

Richardson P.G., Weller E., Lonial S., Jakubowiak A.J., Jagannath S., Raje N.S., et al. **Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma**. Blood. 2010; 116: 679-686.

Rodriguez-Gonzalez A., Lin T., Ikeda A.K., Simms-Waldrip T., Fu C., Sakamoto K.M. **Role of the aggresome pathway in cancer: targeting histone deacetylase 6-dependent protein degradation**. Cancer Res. 2008; 68: 2557-2560.

Rogov V., Dotsch V., Johansen T., Kirkin V. **Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy**. Mol Cell. 2014; 53: 167-178.

San-Miguel J.F., Hungria V.T., Yoon S.S., Beksac M., Dimopoulos M.A., Elghandour A., et al. **Overall survival of patients with relapsed multiple myeloma treated with panobinostat or placebo plus bortezomib and dexamethasone (the PANORAMA 1 trial): a randomised, placebo-controlled, phase 3 trial**. Lancet Haematol. 2016; 3(11): e506-e515.

Sanchorawala V., Brauneis D., Shelton A.C., Lo S., Sun F., Sloan J.M., et al. **Induction Therapy with Bortezomib Followed by Bortezomib-High Dose Melphalan and Stem Cell Transplantation for Light Chain Amyloidosis: Results of a Prospective Clinical Trial**. Biol Blood Marrow Transplant. 2015; 21: 1445-1451.

Saric T., Graef C.I., Goldberg A.L. **Pathway for degradation of peptides generated by proteasomes: a key role for thimet oligopeptidase and other metallopeptidases**. J Biol Chem. 2004; 279: 46723-46732.

Schrader J., Henneberg F., Mata R.A., Tittmann K., Schneider T.R., Stark H., et al. **The inhibition mechanism of human 20S proteasomes enables next-generation inhibitor design**. Science. 2016; 353: 594-598.

Seifert U., Bialy L.P., Ebstein F., Bech-Otschir D., Voigt A., Schroter F. **Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress**. Cell. 2010; 142: 613-624.

Shahnawaz M., Park K.W., Mukherjee A., Diaz-Espinoza R., Soto C. **Prion-like characteristics of the bacterial protein Microcin E492**. Sci Rep. 2017; 7: 45720.

Sonneveld P., Schmidt-Wolf I.G., van der Holt B., El Jarari L., Bertsch U., Salwender H., et al. **Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: results of the randomized phase III HOVON-65/ GMMG-HD4 trial**. J Clin Oncol. 2012; 30: 2946-2955.

Srinivas N.R. **Clinical pharmacokinetics of panobinostat, a novel histone deacetylase (HDAC) inhibitor: review and perspectives**. Xenobiotica. 2017; 47: 354-368.

Suraweera A., Munch C., Hanssum A., Bertolotti A. **Failure of amino acid homeostasis causes cell death following proteasome inhibition**. Mol Cell. 2012; 48: 242-253.

Tekirdag K., Cuervo A.M. **Chaperone-mediated autophagy and endosomal microautophagy: Joint by a chaperone**. J Biol Chem. 2018; 293: 5414-5424.

Walsh D.M., Selkoe D.J. **Oligomers on the brain: the emerging role of soluble protein aggregates in neurodegeneration**. Protein Pept Lett. 2004; 11: 213-228.

Wang J., Fang Y., Fan R.A., Kirk C.J. **Proteasome Inhibitors and Their Pharmacokinetics, Pharmacodynamics, and Metabolism**. Int J Mol Sci. 2021; 22.

Wojcik C. **Regulation of apoptosis by the ubiquitin and proteasome pathway**. J Cell Mol Med. 2002; 6: 25-48.

Wojcik S., Wilk D.S., Lamprecht J., Paweletz N. **Ubiquitin-mediated proteolysis centers in HeLa cells: indication from studies of an inhibitor of the chymotrypsin-like activity of the proteasome**. Eur J Cell Biol. 1996; 71(73): 311-318.

Wolf J.L., Siegel D., Goldschmidt H., Hazell K., Bourquelot P.M., Bengoudifa B.R., et al. **Phase II trial of the pan-deacetylase inhibitor panobinostat as a single agent in advanced relapsed/refractory multiple myeloma**. Leuk Lymphoma. 2012; 53: 1820-1823.

Wooten M.W., Geetha T., Babu J.R., Seibenhener M.L., Peng J., Cox N. **Essential role of sequestosome 1/p62 in regulating accumulation of Lys63-ubiquitinated proteins**. J Biol Chem. 2008; 283:6783-6789.

Wurzer B., Zaffagnini G., Fracchiolla D., Turco E., Abert C., Romanov J., et al. **Oligomerization of p62 allows for selection of ubiquitinated cargo and isolation membrane during selective autophagy**. Elife. 2015; 4: e08941.

Yan J., Seibenhener M.L., Calderilla-Barbosa L., Diaz-Meco M.T., Moscat J., Jiang J., et al. **SQSTM1/p62 interacts with HDAC6 and regulates deacetylase activity**. PLoS One. 2013; 8: e76016.

Yau R.G., Doerner K., Castellanos E.R., Haakonsen D.L., Werner A., Wang N. **Assembly and Function of Heterotypic Ubiquitin Chains in Cell-Cycle and Protein Quality Control**. Cell. 2017; 171: 918-933 e920.

Yu L., Chen Y., Tooze S.A. **Autophagy pathway: Cellular and molecular mechanisms**. Autophagy. 2018; 14: 207-215.

Zhang C., Huang C., Xia H., Xu H., Tang Q., Bi F. **Autophagic sequestration of SQSTM1 disrupts the aggresome formation of ubiquitinated proteins during proteasome inhibition**. Cell Death Dis. 2022; 13: 615.

Review and Research on Cancer Treatment
Volume 9, Issue 1 (2023)

The effectiveness and mechanism of action of N-acetylcysteine in cancer

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ABSTRACT

Introduction: N-acetylcysteine (NAC) is a sulfhydryl substance, a derivative of the amino acid L-cysteine, exhibiting high antioxidant properties. NAC is currently used as a mucolytic and antidote to acetaminophen and as an antioxidant in chronic diseases caused by oxidative stress. Although cancer is characterized by high level of ROS and increased oxidative stress, its use in the treat-ment of this disease is controversial. Due to varying research results, there is ongoing scientific debate whether or not NAC may be effective in treating cancer.

Material and methods: In order to search for scientific articles, the PubMed database was used with the following keywords: N-acetylcysteine, cancer, cancer metastases, antioxidant treatment, mechanism of action, ROS, antioxidant, tumor angiogenesis, antioxidant therapy.

Results: NAC supplementation can reduce tumor cell proliferation, migration, and invasion in various types of cancer. NAC reduces toxicity caused by chemotherapy and radiotherapy in the course of cancer therapy and exerts a preventive effect, hampering the development of cancer. However, caution should be exercised when using NAC in cancer patients, especially with regard to metastases, as NAC was reported to intensify them in some studies. Thus, the results of using NAC in humans may depend on the stage of cancer. Moreover, the reason for the various effects of NAC in cancer treatment seems to be the involvement of this drug in the modulation of signaling pathways that can induce or inhibit cancer.

Summary: The use of NAC in cancer treatment results in outcomes ranging from beneficial to harmful, mainly because ROS can mediate both cancer-promoting and cancer-inhibitory signalling. Negative NAC results were mainly related to metastasis. The effect of NAC may depend on whether the type of cancer depends on ROS signalling for its survival and metastasis. Further research is needed to resolve the role of NAC in cancer treatment.

INTRODUCTION

Reactive oxygen species (ROS) play an important role in physiology, but in large amounts they can lead to oxidative stress. Oxidative stress occurs when ROS levels are excessive and antioxidant levels are relatively scare. ROS in large doses can cause oxidative damage to molecules such as nucleic acids, proteins, lipids, glucose, and consequently the destruction of enzymes and damage to structural protein membranes, gene mutation, and even pro-oncogenic signaling activation. The pathogenesis of several serious human diseases, including cancer, is related to oxidative stress (Radomska-Leśniewska, 2017). Increased oxidative stress can initiate tumor development and cause cancer progression through direct oxidation of macromolecules or aberrant redox signaling (Luo, 2011). Since oxidative stress plays an important role in carcinogenesis and cancer progression, the use of antioxidants to modulate ROS levels in cancer treatment is warranted (Hayes, 2020; Klaunig, 2018). Modulating ROS levels is a promising anticancer strategy and enables the inhibition of carcinogenesis and tumor development induced by oxidative damage and ROS-dependent cell death (Poprac, 2017; Forman, 2021).

N-acetylcysteine (NAC) is a synthetic antioxidant and has strong direct and indirect antioxidant effects. NAC is currently used mainly as a mucolytic and antidote to acetaminophen and as an antioxidant in chronic diseases caused by oxidative stress including lung diseases, cardiovascular diseases, kidney diseases, liver diseases, infectious diseases, psychiatric illness. Although cancer is characterized by increased oxidative stress, the use of NAC in cancer treatment is controversial. Due to varying study results, this review discusses whether NAC can be effective and safe in the treatment of cancer (Tenorio, 2021).

SEARCH STRATEGY AND SELECTION CRITERIA

The aim of the work was to collect data about the mechanism of action of N-acetylcysteine, determine the effectiveness of NAC application in cancer, and safety of using this drug. The authors reviewed information from original articles published up to 2023. The articles were searched in the PubMed database, using the following keyword: N-acetylcysteine, cancer, cancer metastases, antioxidant treatment, mechanism of action, ROS, antioxidant, tumor angiogenesis, antioxidant therapy. Manually selected materials related to the topic were added.

RESULTS

ROS AND OXIDATIVE STRESS

ROSs are natural products of the oxygen metabolism of the cells (Augustyniak, 2010). During cellular metabolism in mitochondria, molecular O_2 is converted into H_2O in the oxidative phosphorylation process resulting in ROS generation: superoxide radical (O^{2-} •), hydrogen peroxide (H_2O_2), and the hydroxyl radical (• OH). H_2O_2 is also produced by peroxisomes (Rushworth, 2014).

The level of ROSs depends on both endogenous ROS formation and exposure to exogenous ROSs. The impact of ROS on physiology depends on their concentration. A low level of ROS is necessary for the proper functioning of the organism. They participate in gene expression modulation, intracellular signal transduction, cell proliferation, transcription, and apoptosis (Ushio-Fukai, 2008; Polsjak, 2013), as well as Ca^{2+} circulation and protein phosphorylation. ROS at a low level activates some transcription factors, including nuclear factor κB (NFκB) or activator protein 1 (AP-1) (Ushio-Fukai, 2008). The proper course of inflammatory and angiogenesis processes also depends on ROS (Bir, 2013; Nijmeh, 2010). ROS in high levels act to detriment the body, being cytotoxic and mutagenic to cells, leading to apoptosis and cell death (Manea, 2010). The body's defense against ROS toxicity is provided by antioxidants, substances that neutralize free radicals or their actions.

Oxidative stress means an imbalance between the level of ROS production and antioxidant mechanisms). It leads to disorders of cellular metabolism and biological functions of cells and the organism, as a result of damage of cellular elements, including: DNA, proteins and lipids (Rushworth, 2014; Ames, 1993).

Many transcription factors can be induced by oxidative stress. One of them is nuclear factor erythroid 2-related factor 2 (Nrf2), which regulates the expression of molecules performing antioxidant functions in the cell (Gorrini, 2013). Nrf2 protects cells against oxidative or electrophilic stress by activating downstream target genes and enzymes such as heme oxygenase 1 (HO-1), NAD(P)H oxidase 1 (NOX1), NAD(P)H quinone oxidoreductase 1 (NQO1), glutamate cysteine ligase catalytic subunit (GCLC), glutamate ligase modifier subunit cysteine (GCLM), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Suzuki, 2017). The antioxidant defense system, in addition to the these enzymes, include vitamin C and vitamin E, thiols or sulfhydryl-containing compounds such as glutathione (GSH) and thioredoxin. GSH contains a free sulfhydryl group, thanks to which it has an antioxidant effect and is a source of reducing equivalents that remove harmful ROS (Rushworth, 2014). In addition to its antioxidant effects, GSH exerts a positive effect through protein thiolation, drug detoxification, and regulation of signal transduction modulated by oxidation-reduction reactions (Frye, 2019, de Andrade, 2015, Rushworth, 2014).

ROS IN CANCER

Cancer cells produce high levels of ROS which participate in the generation of oxidative stress leading to abnormal redox signaling. Oxidative stress in turn, may contribute to the development of cancer. Increased oxidative stress in cancer cells is responsible for the glycolytic and catabolic state. Catabolites, including lactate, are released there supporting mitochondrial metabolism through mitochondrial heterogeneity (Martinez-Outschoorn, 2016). Mitochondrial heterogeneity contributes to the increase of oxidative stress in cancer cell, their proliferation, tumor growth, and metastasis (Dhouib, 2016).The increased levels of ROS exert various effects on cancer cells. On the one hand, in huge amounts, they produce anticancer effects through oxidative damage and ROS-dependent death signaling. ROS increases gene mutation and pathological inflammation by directly oxidizing macromolecules such as nucleic acids, proteins, lipids and glucose (Zhong, 2015). On the other hand, lower levels of ROS play a key role during tumorigenesis and promotes cancer cell proliferation and cancer progression. Cancer ROS, especially H_2O_2 and $O^{2\cdot-}$, can act as signaling molecules, causing malfunction of various signaling pathways. In cancer cells ROSs are involved in upregulation of HIF-1α and VEGF protein expression through activation of PI3K/Akt/p706K pathway or MEK/ERK pathway (Ushio-Fukai, 2008). The high level of VEGF and MMP9, stimulated by NOX 1, is a characteristic feature of cancer cells. Therefore ROS induces angiogenesis in tumor which contribute to their development as well (Radomska-Leśniewska, 2016).

N-ACETYLCYSTEINE

NAC contains the amino acid L-cysteine (with sulphhydryl group) and acetyl group attached to the amino (NH_2) group and it possesses high antioxidant capacities. It was first introduced as a mucolytic drug in respiratory diseases (e.g. cystic fibrosis) in 1963. Then it was used in paracetamol poisoning. In cancer

biology and immuno-oncology, NAC was used as a direct scavenger of reactive oxygen species (especially hydrogen peroxide) and as an antioxidant. NAC is safe and well tolerated, even at high doses (Ooi, 2018). It can be administered orally, intravenously, and by inhalation. After oral administration, its maximum plasma concentration (C_{max}) occurs up 2 hours (Dodd, 2008). NAC crosses the membrane and crosses the blood-brain barrier, depending on the dose and method of administration (Hara, 2017). The effects of NAC are attributed to its thiol modulation in cells (Aldini, 2018; Bansal, 2018; Cheng, 2023). The main products of NAC metabolism after complete metabolism, are cysteine, cystine, inorganic sulfate, and glutathione (Prescott, 1989). The bioavailability of NAC is approximately 10% and only a small amount reaches the plasma and tissues (Ezerina, 2018). After intravenous administration of NAC at a dose of 150 mg/kg over 15 min, the C_{max} of NAC averaged 554 mg/l (De Andrade, 2015). Since the bioavailability of NAC is relatively low NAC is used in high concentrations in studies (Cheng, 2023).

MECHANISM OF ACTION

NAC may act through several possible mechanisms including its key role for the intracellular role in GSH biosynthesis, as well as its antioxidant function through nucleophilic character – an extracellular scavenger. L-cysteine availability in the cell is rate-limiting for GSH synthesis, and NAC is essentially a prodrug that is converted to l-cysteine. L-cysteine, a precursor of reduced GSH, is a major endogenous antioxidant (Sadowska, 2005). NAC activity increases the concentration of GSH within the cells so it is able to restore disturbed antioxidant levels. It is known that oxidative stress and inflammation can be the cause of diminished levels of GSH. That is why NAC can normalize the disturbed redox status of the cell and modulates redox sensitive cell signaling and transcription pathways (de Andrade, 2015).

Moreover, the high antioxidant properties of NAC are due to the sulfhydryl compound, which enables direct scavenging of ROS such as superoxide radical, hydrogen peroxide, and hydroxyl radical. NAC may also act as a direct scavenger of peroxynitrite or related pathways. As mentioned above, the role of ROS depends mainly on their quantity, small amounts are necessary and beneficial for the body, while too large amounts of ROS are toxic (Aldini, 2018).

It should also be emphasized that the effect of NAC may sometimes be opposite, i.e. it may have pro-oxidant properties as a result of the auto-oxidation process causing the formation of H_2O_2 in the presence of O_2 (Lee, 2011).

NAC was reported to inhibit several pro-inflammatory and antiapoptotic pathways such as nuclear factor kappa B (NFκB), p38 MAP kinase, SAPK/INK, c-Fos, c-Jun N-terminal kinase pathways, and cyclin inhibitors (Radomska-Leśniewska, 2010; Radomska-Leśniewska, 2016; Sadowska, 2005; Zafarullah 2003) (Fig. 1).

Therefore NAC has been shown to inhibit various anti-inflammatory cytokines such as interleukin 8 (IL-8), IL-6, and tumor necrosis factor α (TNF-α) (Radomska-Leśniewska, 2010; Radomska-Leśniewska, 2006; Maher, 2007). A reduction in collagen synthesis and fibroblast proliferation was also demonstrated by NAC (Ask, 2006) (Fig. 1).

Since cancer is associated with impaired, abnormally high angiogenesis, NAC may be a potential drug that normalizes this process. NAC has been proven to effectively reduce angiogenesis-induced vascular endothelial growth factor (VEGF) (Ushio-Fukai, 2002), endothelial cell invasion, and *in vitro* angiogenesis by inhibiting metalloproteinase (MMP) activity (Cai, 1999). These studies are consistent with the results of our group, in which we presented the inhibition of MMP9 and the pro-angiogenic intercellular adhesion molecule-1 (ICAM-1) by this drug (Radomska-Leśniewska, 2006; Radomska -Leśniewska, 2010). NAC inhibited the expression of the most powerful stimulator of angiogenesis – VEGF in ras-transformed cancer cells (Nijmeh, 2010). It is also known that the above-mentioned drug has a cytoprotective effect on endothelial cells (Aluigi, 2000) (Fig. 1).

The mucolytic effect of NAC consists of breaking the disulfide bonds of highly cross-linked mucus glycoproteins (mucins), thus reducing the viscosity of mucus (Aldini, 2018). As an antidote to acetaminophen poisoning, NAC restores the hepatic GSH pool depleted in the drug detoxification process. GSH, in turn, neutralizes the N-acetyl-p-benzoquinoneimine (NAPQI) – the harmful metabolite of acetaminophen, and scavenges reactive oxygen and nitrogen species (Aldini, 2018, Rushworth, 2014).

NAC IN CANCER STUDIES

The effect of ROS (peroxide and H_2O_2) on cancer has been shown to be concentration-dependent. As it was mentioned above at low levels, superoxide/H_2O_2 induces cell proliferation and promotes cancer formation and progression while high levels of ROS can be cytotoxic to cancer cells and inhibit metastasis. Therefore, the impact of antioxidants may also exert various effects on cancer. Antioxidants can generally inhibit the formation and progression of cancer but potentially may contribute to the development of metastases (Hayes, 2020; Gill, 2016).

NAC has been extensively studied, due to its unique biological properties, as an agent in the prevention and treatment of cancer, as well as an agent to counteract the effects of chemotherapy and radiotherapy.

THERAPEUTIC POTENTIAL OF NAC

NAC was reported to enhance cancer cell apoptosis, reduce catabolism, mitochondrial dysfunction, and inflammatory, and inhibits oxidative stress mediators (Dhouib, 2016). Lung cancer studies revealed NAC abilities to detoxify chemicals, scavenge radicals and protect against DNA damage. NAC combined with epigallocatechin-3-gallate (EGCG), the main green tea polyphenols, form an adduct that may enhance the killing of cancer cells (Lambert, 2008) (Tab. 1). Reports emphasize the dual role of ROS and GSH in cancer initiation and progression (Fendt, 2020; Bansal, 2018). NAC treatment was reported to alleviate of ROS in the tumor microenvironment in triple-negative breast cancer (Kwon, 2021) Table 1). NAC administered with IL-2 synergistically enhanced the level of GSH and thus increased the effectiveness of interleukin-2/lymphokine activating therapy (Yim, 1994). In a human pilot study determining the antiproliferative effects of NAC on breast cancer NAC markedly reduces monocarboxylate transporter 4 (MCT4) transporter proteins from being utilized to import energy as lactate to cancer cells. MCT4 is considered a marker of aggressive cancer behavior with poor overall survival. NAC administration was associated with reduced breast cancer cell proliferation, oxidative stress, and inhibition of breast cancer stromal cell metabolism (Monti, 2017) (Tab. 1). A study in a mouse model of melanoma showed that NAC pretreatment had a beneficial effect by blocking the formation of 8-oxoguanine in mouse skin after neonatal UV treatment and delaying the onset of melanoma (Cotter, 2007) (Tab. 1). Similarly, good results were obtained in a study of patients with a history of melanoma and/or atypical nevus, where NAC reduced oxidative stress and glutathione deficiency in nevus caused by UV radiation (Goodson, 2009). NAC in glioblastoma multiforme may prevent proliferation, migration, and invasion in an antioxidant-independent manner by modulating Notch2 signaling (Deng, 2019). In an oncogenic KRAS ^{G12D}-driven mouse model (increased NADPH oxidase and decreased NRF2), lung adenocarcinoma attenuation was associated with ROS suppression by NAC (Song, 2018). It was also reported that NAC sensitizes pancreatic cancer cells to gemcitabine (Qanungo, 2014). NAC inhibited prostate cancer cell growth and prevented adhesion and invasion to remote locations in prostate cancer cell study (Lee, 2011) (Tab. 1).

The results of studies on the effect of NAC on cancer at the metastatic stage are ambiguous. NAC was shown to inhibit lung metastasis by Tigar/null pancreatic cancer cells. Furthermore, mitochondrially targeted antioxidant mito-TEMPO inhibited lung metastasis of orthotopically injected MDA-MB-231 breast cancer cells in immunodeficient mice (Cheng, 2023) (Tab. 1). However in a mouse model of lung cancer NAC increases lung cancer metastasis (Breau, 2019; Gill, 2016) which was explained by the reduction of oxidative stress in metastatic tumors and development in distant places (Sayin, 2014) (Tab. 1). It was also shown that vitamin E and NAC enhanced cancer cell proliferation by reducing ROS and diminishing p53 in mouse and human lung cancer cells (Sayin, 2014). NAC cultured with human melanoma cells resulted in increased proliferation and migration (Piskounova, 2015) (Tab. 1). Moreover, NAC administered in drinking water enhanced metastasis spread in a murine melanoma model (Le Gal, 2015). Wiel et al. reported that NAC and vitamin E increase metastasis to the liver, kidney, heart, and rib cage of lung cells harboring oncogenic K- RAS^{G12D} (Wiel, 2019). These antioxidants increased metastasis by reducing the level of ROS and free heme, which led to the stabilization of the transcription factors BTB (Broad-Complex, Tramtrack and Bric a brac) and CNC1 homologs (BACH1), whose function is to increase glucose uptake, glycolysis, and lactate secretion via Mct1 (Wiel, 2019) (Tab. 1, Figure 1). BACH1 is considered to be one of the master regulators of metastasis (Lee, 2011) and increases the expression of metastasis-related genes such as MMP1, MMP3, CXCR4 (C-X-C chemokine receptor type 4), CTGF (Connective tissue growth factor), PGK2 (Phosphoglycerate Kinase 2), and ROBO1(Roundabout Guidance

Receptor 1) (Liang, 2012) (Fig. 1). Moreover, it was also demonstrated that high NAC doses caused increased metastasize, increased ROS production, and increased NRF2 nuclear translocation (Obrador, 2022). NRF2 a is key regulator of expression of molecules performing antioxidant functions in the cell but high NRF2 levels can promote metastasis (Becker, 2023). As was reported, Nrf2 plays a crucial role in the metastasis of cervical cancer by enhancing EMT (Zhang, 2023).

Reports have shown the enhancing effect of NAC on anti-tumor NK cells modified with chimeric antigen receptor (CAR) (Klopotowska, 2022). It was also revealed that NAC also stimulated the antitumor function of exhausted T lymphocytes. The mechanisms of stimulation of NAC action in immune cells are similar to those suggested in cancer cells (Scheffel, 2018).

ANTIANGIOGENIC POTENTIAL OF NAC

It was shown that neoplastic diseases are characterized by high level of pathologic angiogenesis because tumor needs blood supply for growth and development (Radomska-Leśniewska, 2016). As was mentioned above NAC exerted anti-angiogenic properties which were revealed by inhibiting neovascularization both *in vivo* and *in vitro* studies, but also by inhibiting pro-angiogenic markers such as MMP9, Il-8, ICAM-1, and VEGF (Radomska-Leśniewska 2010, Sadowska, 2007, Radomska-Leśniewska, 2006). The mechanism of the antiangiogenic effect of NAC is based on the reduction of the level of HIF-1α by this antioxidant (Gao, 2007). The antiangiogenic activity of NAC promotes the inhibition of tumor growth and development.

Table 1. NAC effect in the study of various types of cancer

Type of cancer in the study	NAC effect	Source
Beneficial effects		
Lung cancer	Enhances EGCG-mediacted cel depletion	Lambert, 2008
Triple-negative breast cancer	Inhibits ROS-mediated signaling – possibly beneficial effect	Kwon, 2021
Breast cancer	Reduces MCT4 and cel proliferation	Monti, 2017
Melanoma	Blocks formation of 8-oxoguanine, reduces oxidative stress – delayed onset of UV-induced melanoma	Cotter, 2007 Goodson, 2009
Glioblastoma	Modulates Notch 2 signaling, prevents cancer proliferation and invasion	Deng, 2019
Lung adenocarcinoma	Suppresses ROS and cancer development	Song, 2018
Pancreatic cancer	Sensitizes to gemcitabine	Qanungo, 2014
Prostate cancer	Prevents growth, adhesion and invasion	Lee, 2011
Tigar/null pancreatic cancer	Inhibits lung metastasis	Cheng, 2023
Melanoma	Stimulates anti-tumor cytotoxic T cells	Scheffel, 2018
Harmful effects		
Lung cancer	Increases metastases	Gill, 2016 Breau, 2019 Wiel, 2019
Lung cancer	With vitamin E increases proliferation, reduces ROS and p53 levels.	Sayin, 2014
Melanoma	Increases proliferation and migration.	Piskounova, 2015 Le Gal, 2015
Melanoma	Increases metastases and Nrf2 nuclear translocation	Obrador, 2022
Breast cancer	Reduces ROS levels, increases expression of metastasis-related genes	Liang, 2012

ECGG – epigallocatechin-3-gallate MCT4 – monocarboxylate transporter 4

PREVENTIVE POTENTIAL OF NAC

It is suggested that NAC and other antioxidants may potentially prevent cancer, because DNA damage caused by oxidative stress and genome instability may lead to cancerous transformation (Radomska-Leśniewska, 2016). A clinical trial in which patients were given 1,800 mg of NAC daily showed that this

was a sufficient dose to prevent cancer and lower markers of oxidative stress (Block, 2008). The "Euro-scan" study, in which only 600 mg of NAC was administered daily, did not show positive results in patients with head, neck and lung cancer, probably due to too low dose of the drug (van Zandwijk, 2000). Then preventive capabilities of NAC can be dose-depended. Many other studies on the prevention and anticancer and antiangiogenic effects of NAC have been performed *in vitro* and in animal models. These studies demonstrated the effectiveness of NAC in cancer prevention (De Flora, 1996; Reliene, 2006). NAC administered in drinking water inhibited the incidence and proliferation of tumors in lymphoma-bearing mice with the ataxia telangiectasia mutation (Reliene, 2006). Moreover, inhibition of metastasis and cell proliferation has been described in a tumor angiogenesis model in athymic breast cancer-bearing mice treated by NAC (Agarwal, 2004). NAC was shown to be a potential pharmacological agent for the prevention and treatment of cervical cancer. In this study, NAC promoted apoptosis in HPV-positive cells and effectively reduced the proliferation of HPV-positive cells by inhibiting cIAP2 and HIF-1α (Guo, 2023).

Gao et al. found that NAC (applied in drinking water) inhibited three *in vivo* mouse tumor models. The NAC effect was mediated by inhibition of HIF-1 levels in a MYC-dependent human B lymphoma model (Gao, 2007). A similar anticancer/antiangiogenic NAC effect was obtained in multiple studies performed on cell lines including human melanoma (Cotter, 2007), lung cancer cells (H1299) (Liu, 2012), androgen-independent prostate carcinoma PC-3 cells (Lee 2011), and others (Tab. 1).

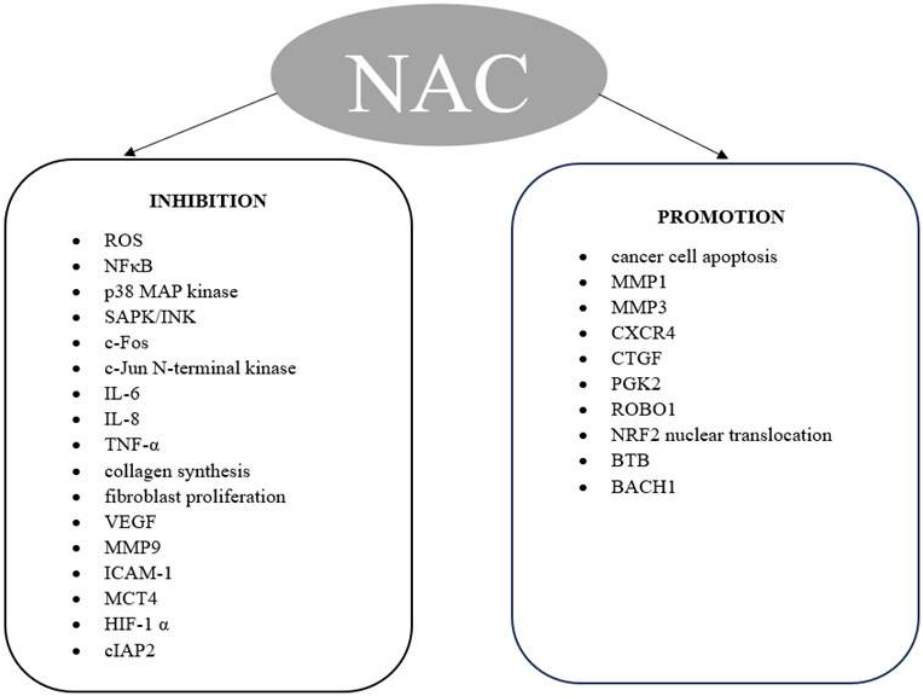

Figure 1. The Effect of NAC on molecular pathways components and molecules levels

RADIO- AND CHEMOPREVENTIVE ACTIVITY OF NAC

Although radiotherapy (RT) and chemotherapy is an important components of cancer treatment, they induces adverse tissue reactions in the around of cancer tissue. Therefore, radioprotective agent is needed to secure normal tissues.

Barlaz et al. reported a radioprotective effect of NAC on RT-induced cardiac damage in rats for the acute term. Results supporting cardiac injury were observed in the electrocardiogram. Furthermore, cytokine levels and oxidative stress were also significantly increased. NAC was reported to reduce these signs of cardiac damage and, therefore may be a potential radioprotector that is capable of preventing cardiac damage (Barlaz, 2020).

NAC administered at a dose of 2,400 mg by nebulization for 8 weeks to patients after radiotherapy with head and neck cancer improved their quality of life expressed by a greater reduction in the use of analgesic drugs and improved xerostomia (Won, 2020). Similar results (improved xerostomia and saliva thickening) were obtained in a study of patients with head and neck cancer who had rinsed their mouths with NAC

(2,500 mg daily) before and after radiotherapy (Sio, 2019). Moreover, in patients with the same cancer, transtympanic NAC injections performed before radiotherapy prevented cisplatin-induced ototoxicity (Yoo, 2014).

A meta-analysis study revealed that NAC and other antioxidants inhibit the toxicity caused by cancer therapy in most cases (Block, 2008). In a group of 40 children with acute lymphoblastic leukemia after chemotherapy/radiotherapy, vitamin E and NAC were proven to be effective as adjuvant antioxidant therapy. The toxicity of chemotherapy and radiotherapy was significantly reduced as measured by reduced levels of malondialdehyde, increased levels of glutathione peroxidase, and reduced incidence of toxic hepatitis (Al-Tonbary, 2009).

SUMMARY AND CONCLUSION

NAC supplementation can reduce tumor cell proliferation, migration, and invasion in various types of cancer. It also has chemopreventive properties, eliminating the negative effects of chemotherapy and radiotherapy. The preventive effect of NAC is also known. However, caution should be exercised when using NAC in cancer patients, especially with regard to metastases, as NAC was reported to intensify them in some studies. Thus, results of using of NAC in humans, may depend on stage of cancer as well as tumor type and organ subject to colonization. Moreover, the reason for the various effects of NAC in cancer treatment seems to be the involvement of this drug in the modulation of signaling pathways that can induce or inhibit cancer. Further research is needed to resolve the role of NAC in cancer treatment.

References

Aldini G., Altomare A., Baron G., Vistoli G., Carini M., Borsani L., et al. **N-Acetylcysteine as an antioxidant and disulphide breaking agent: The reasons why**. Free Radic Res. 2018; 52: 751-762.

Al-Tonbary Y., Al-Haggar M., El-Ashry R., El-Dakroory S., Azzam H., Fouda A. **Vitamin E and N- acetylcysteine as antioxidant adjuvant therapy**. Adv Haematol. 2009; 4: 1-5.

Aluigi M.G., De Flora S., D'Agostini F., Albini A., Fassina G. **Antiapoptotic and antigenotoxic effects of N-acetylcysteine in human cells of endothelial origin**. Anticancer Res. 2000; 20(5A): 3183-7.

Ames B.N., Shigenaga M.K., Hagen T.M. **Oxidants, antioxidants, and the degenerative diseases of aging**. Proc Natl Acad Sci U S A. 1993; 90(17): 7915-7922.

Ask K., Martin G.E., Kolb M., Gauldie J. **Targeting genes for treatment in idiopathic pulmonary fibrosis: challenges and opportunities, promises and pitfalls**. Proc Am Thorac Soc. 2006; 3: 389-393.

Augustyniak A., Bartosz G., Cipak A., Duburs G., Horáková L., Luczaj W., et al. **Natural and synthetic antioxidants: an updated overview**. Free Radic Res. 2010; 44(10):1216-62.

Bansal A., Simon M.C. **Glutathione metabolism in cancer progression and treatment resistance**. J Cell Biol. 2018; 217(7): 2291-2298.

Barlaz Us S., Vezir O., Yildirim M., Bayrak G., Yalin S., Balli E., et al. **Protective effect of N-acetyl cysteine against radiotherapy-induced cardiac damage**. Int J Radiat Biol. 2020; 96(5): 661-670.

Becker A.L., Indra A.K. **Oxidative Stress in Melanoma: Beneficial Antioxidant and Pro-Oxidant Therapeutic Strategies**. Cancers (Basel). 2023; 15(11): 3038.

Bir S.C., Shen X., Kavanagh T.J., Kevil C.G., Pattillo C.B. **Control of angiogenesis dictated by picomolar superoxide levels**. Free Radic Biol Med. 2013; 63: 135-42.

Block K.I., Koch A.C., Mead M.N., Tothy P.K., Newman R.A., Gyllenhaal C. **Impact of antioxidant supplementation on chemotherapeutic toxicity: a systematic review of the evidence from randomized controlled trials**. Int J Cancer. 2008; 123(6): 1227-39.

Breau M., Houssaini A., Lipskaia L., Abid S., Born E., Marcos E., et al. **The antioxidant N-acetylcysteine protects from lung emphysema but induces lung adenocarcinoma in mice**. JCI Insight. 2019; 4(19): e127647.

Cai T., Fassina G., Morini M., El-Dakroory S., Azzam H., Fouda A. **N-acetylcysteine inhibits endothelial cell invasion and angiogenesis**. Lab Invest. 1999; 79: 1151-1159.

Cheng G., Hardy M., Kalyanaraman B. **Antiproliferative effects of mitochondria-targeted N-acetylcysteine and analogs in cancer cells**. Sci Rep. 2023; 13(1): 7254.

Cotter M.A., Thomas J., Cassidy P., Robinette K., Jenkins N., Florell S.R., et al. **N-Acetylcysteine Protects Melanocytes against Oxidative Stress/Damage and Delays Onset of Ultraviolet-Induced Melanoma in Mice**. Clin Cancer Res. 2007; 13: 5952-5958.

De Andrade K.Q., Moura F.A., Dos Santos J.M., De Araújo O.R., De Farias Santos J.C., Goulart M.O.F. **Oxidative stress and inflammation in hepatic diseases: Therapeutic possibilities of N-Acetylcysteine**. Int J Mol Sci. 2015; 16: 30269-30308.

De Flora S., D'Agostini F., Masiello L., Giunciuglio D., Albini A. **Synergism between N-acetylcysteine and doxorubicin in the prevention of tumorigenicity and metastasis in murine models**. Int J Cancer. 1996; 67: 842-8.

Deng J., Liu A.D., Hou G.Q., Zhang X., Ren K., Chen X.Z., et al. **N-acetylcysteine decreases malignant characteristics of glioblastoma cells by inhibiting Notch2 signaling**. J Exp Clin Cancer Res. 2019; 38(1): 2.

Devasagayam T.P.A., Tilak J.C., Boloor K.K., Sane K.S., Ghaskadbi S.S., Lele R.D. **Free radicals and antioxidant in human health: current status and future prospects**. JAPI. 2004; 52: 794-804.

Dhouib I.E., Jallouli M., Annabi A., Gharbi N., Elfazaa S., Lasram M.M. **A minireview on N-acetylcysteine: An old drug with new approaches**. Life Sci. 2016; 151: 359-363.

Dodd S., Dean O., Copolov D.L., Malhi G.S., Berk M. **N-acetylcysteine for antioxidant therapy: Pharmacology and clinical utility**. Expert Opin Biol Ther. 2008; 8: 1955-1962.

Ezerina D., Takano Y., Hanaoka K., Urano Y., Dick T.P. **N-Acetyl cysteine functions as a fast-acting antioxidant by triggering intracellular h2s an sulfane sulfur production**. Cell Chem Biol. 2018; 25: 447-459.

Fendt S.M., Frezza C., Erez A.. **Targeting metabolic plasticity and flexibility dynamics for cancer therapy**. Cancer Discov. 2020; 10(12): 1797-1807.

Forman H.J., Zhang H. **Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy**. Nat Rev Drug Discov. 2021; 20: 689-709.

Frye R.E., Andrus J.P., Lemley K.V., De Rosa S.C., Ghezzi P., Holmgren A., et al. **Pharmacology, Formulations, and Adverse Effects.** In: Frye R.E., Berk M. (Editors) **The Therapeutic Use of N-Acetylcysteine (NAC) in Medicine.** 2019; Springer Nature (Singapore); ISBN: 978-981-10-5311-5.

Gao P., Zhang G., Dinavahi R., Li F., Xiang Y., Raman V., et al. **HIF-dependent anti-tumorigenic effect of anti-oxidants *in vivo***. Cancer Cell. 2007; 12(3): 230-8.

Gill J.G., Piskounova E., Morrison S.J. **Cancer, oxidative stress, and metastasis**. Cold Spring Harbor Symp. Quant Biol. 2016; 81: 163-175.

Goodson A.G., Cotter M.A., Cassidy P., Wade M., Florell S.R., Liu T., et al. **Use of Oral N-Acetylcysteine for Protection of Melanocytic Nevi against UV-Induced Oxidative Stress: Towards a Novel Paradigm for Melanoma Chemoprevention**. Clin Cancer Res. 2009; 15: 7434-7440.

Gorrini C., Harris I.S., Mak T.W. **Modulation of oxidative stress as an anticancer strategy**. Nat Rev Drug Discov. 2013; 12: 931-947.

Guo J., Wang J. ***N*-Acetyl-L-Cysteine Reduces Cervical Carcinogenesis by Promoting Apoptosis**. Drugs in R&D. 2023; 23: 165-174.

Hara Y., McKeehan N., Dacks P.A., Fillit H.M. **Evaluation of the neuroprotective potential of N-acetylcysteine for prevention and treatment of cognitive aging and dementia**. J. Prev. Alzheimers Dis. 2017; 4: 201-206.
Hayes J.D., Dinkova-Kostova A.T., Tew K.D. **Oxidative stress in cancer**, Cancer Cell. 2020; 38(2): 167-197.

Klaunig, J.E. **Oxidative Stress and Cancer**. Curr Pharm Des. 2018; 24: 4771-4778.

Klopotowska M., Bajor M., Graczyk-Jarzynka A., Kraft A., Pilch Z., Zhylko A., et al. **PRDX-1 supports the survival and antitumor activity of primary and CAR-modified NK cells under oxidative stress**. Cancer Immunol Res. 2022; 10(2): 228-244.

Kwon Y. **Possible beneficial effects of N-acetylcysteine for treatment of triple-negative breast cancer**. Antioxidants. 2021; 10(2): 169.

Lambert J.D., Sang S., Yang C.S. **N-Acetylcysteine enhances the lung cancer inhibitory effect of epigallocatechin-3-gallate and forms a new adduct**. Free Radic Biol Med. 2008; 44(6): 1069-1074.

Le Gal K., Ibrahim M.X., Wiel C., Sayin V.I., Akula M.K., Karlsson C., et al. **Antioxidants Can Increase Melanoma Metastasis in Mice**. Sci Transl Med. 2015; 7(308): 308re8.

Lee S.J., Noh H.J., Sung E.G., Song I.H., Kim J.Y., Kwon T.K. , et al. **Berberine sensitizes TRAIL-induced apoptosis through proteasome-mediated downregulation of c-FLIP and Mcl-1 proteins**. Int J Oncol. 2011; 38: 485-492.

Liang Y., Wu H., Lei R., Chon R.A., Wei Y., Lu X., et al. **Transcriptional network analysis identifies BACH1 as a master regulator of breast cancer bone metastasis**. J Biol Chem. 2012; 287: 33533-33544.

Liu C., Liu H., Li Y., Wu Z., Zhu Y., Wang T., et al. **Intracellular glutathione content influences the sensitivity of lung cancer cell lines to methylseleninic acid**. Mol Carcinog. 2012; 51(4): 303-14.

Maher T.M., Wells A.U., Laurent G.J**. Idiopathic pulmonary fibrosis: multiple causes and multiple mechanism?** Eur Respir J. 2007; 30: 835-839.

Manea A. **NADPH oxidase-derived reactive oxygen species: involvement in vascular physiology and pathology**. Cell Tissue Res. 2010; 342: 325-39.

Martinez-Outschoorn U.E., Peiris-Pages M., Pestell R.G., Sotgia F., Lisanti M.P. **Cancer metabolism: A therapeutic perspective**. Nat Rev Clin Oncol. 2016; 14: 11-31.

Monti D., Sotgia F., Whitaker-Menezes D., Tuluc M., Birbe R., Berger A., et al. **Pilot study demonstrating metabolic and anti-proliferative effects of in vivo anti-oxidant supplementation with N-Acetylcysteine in Breast Cancer**. Semin Oncol. 2017; 44(3): 226-232.

Nijmeh J., Moldobaeva A., Wagner E.M. **Role of ROS in ischemia-induced lung angiogenesis**. Am J Physiol Lung Cell Mol Physiol. 2010; 299(4): L535-41.

Obrador E., Salvador-Palmer R., López-Blanch R., Oriol-Caballo M., Moreno-Murciano P., Estrela J.M. **N-Acetylcysteine Promotes Metastatic Spread of Melanoma in Mice**. Cancers. 2022, 14: 3614.

Ooi S.L., Green R., Pak S.C. **N-Acetylcysteine for the treatment of psychiatric disorders: A review of current evidence**. BioMed Res Int. 2018; 2018: 8.

Piskounova E., Agathocleous M., Murphy M.M., Hu Z., Huddlestun S.E., Zhao Z., et al. **Oxidative Stress Inhibits Distant Metastasis by Human Melanoma Cells**. Nature. 2015; 527: 186-191.

Polsjak B., Suput D., Milisav I. **Achieving the balance beetween ROS and antioxidants: when to use the synthetic antioxidants**. Oxid Med Cell Longev .2013; 2013:956792.

Poprac P., Jomova K., Simunkova M., Kollar V., Rhodes C.J., Valko M. **Targeting Free Radicals in Oxidative Stress-Related Human Diseases**. Trends Pharmacol Sci. 2017; 38: 592-607.

Prescott L.F., Donovan J.W., Jarvie D.R., Proudfoot A.T. **The disposition and kinetics of intravenous N-acetylcysteine in patients with paracetamol overdosage**. Eur J Clin. Pharmacol. 1989; 37: 501-506.

Qanungo S., Uys J.D., Manevich Y., Distler A.M., Shaner B., Hill E.G., et al. **N-acetyl-L-cysteine sensitizes pancreatic cancers to gemcitabine by targeting the NFκB pathway**. Biomed Pharmacother. 2014; 68(7): 855-864.

Radomska-Leśniewska D.M., Bałan B.J., Skopiński P**. Angiogenesis modulation by exogenous antioxidants**. Cent Eur J Immunol. 2017; 42(4): 370-376.

Radomska-Leśniewska D.M., Hevelke A., Skopiński P., Bałan B., Jóźwiak J., Rokicki D., et al. **Reactive oxygen species and synthetic antioxidants as angiogenesis modulators: Clinical implications**. Pharmacol Rep. 2016; 68(2): 462-71.

Radomska-Leśniewska D.M., Sadowska A.M., Van Overveld F.J., Demkow U., Zieliński J., De Backer W.A. **Influence of N-acetylcysteine on ICAM-1 expression and IL-8 release from endothelial and epithelial cells**. J Physiol Pharmacol. 2006; 57: 325-334.

Radomska-Leśniewska D.M., Skopińska-Różewska E., Jankowska-Steifer E., Sobiecka M., Sadowska A.M., Hevelke A., et al. **N-acetylcysteine inhibits IL-8 and MMP-9 release and ICAM-1 expression by bronchoalveolar cells from interstitial lung disease patients**. Pharmacol Rep. 2010; 62: 131-138.

Reliene R., Schiestl R. **Antioxidant N-acetyl cysteine reduces incidence and multiplicity of lymphoma in Atm deficient mice**. DNA Repair. 2006; 5: 852-9.

Rushworth G.F., Megson I.L. **Existing and potential therapeutic uses for N-acetylcysteine: The need for conversion to intracellular glutathione for antioxidant benefits**. Pharmacol Ther. 2014; 141(2): 150-159.

Sadowska A.M., Manuel-Y-Keenoy B., De Backer W.A. **Antioxidant and anti-inflammatory efficacy of NAC in the treatment of COPD: discordant in vitro and in vivo dose-effects: a review**. Pulm Pharmacol Ther. 2007; 20(1): 9-22.

Sayin V.I., Ibrahim M.X., Larsson E., Nilsson J.A., Lindahl P., Bergo M.O. **Antioxidants accelerate lung cancer progression in mice**. Sci Transl Med. 2014; 6(221): 221ra15.

Scheffel M.J., Scurti G., Wyatt M.M., Garrett-Mayer E., Paulos C.M., Nishimura M. I., et al. **N-acetyl cysteine protects anti-melanoma cytotoxic T cells from exhaustion induced by rapid expansion via the downmodulation of Foxo1 in an Akt-dependent manner**. Cancer Immunol. Immunother. 2018; 67 (4): 691-702.

Sio T.T., Blanchard M.J., Novotny P.J., Patel S.H., Rwigema J.C.M., Pederson L.D., et al. **N-acetylcysteine rinse for thick secretion and mucositis of head and neck chemoradiotherapy (Alliance MC13C2): A double-blind randomized clinical trial.** Mayo Clin Proc. 2019; 94(9):1814-1824.

Song N.Y., Zhu F., Wang Z., Willette-Brown J., Xi S., Sun Z., et al. **IKKα inactivation promotes Kras-initiated lung adenocarcinoma development through disrupting major redox regulatory pathways**. Proc Natl Acad Sci U S A. 2018; 115(4): E812–e821.

Suzuki T., Yamamoto M. **Stress-sensing mechanisms and the physiological roles of the Keap1-Nrf2 system during cellular stress**. J Biol Chem. 2017; 292(41): 16817-16824.

Tenório M.C.D.S., Graciliano N.G., Moura F.A., Oliveira A.C.M., Goulart M.O.F. ***N*-Acetylcysteine (NAC): Impacts on Human Health**. Antioxidants (Basel). 2021; 10(6):967-1001.

Ushio-Fukai M., Nakamura Y. **Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy**. Cancer Lett. 2008; 266(1): 37-52.

Ushio-Fukai M., Tang Y., Fukai T., et al. **Novel role of gp91(phox)-containing NAD(P)H oxidase in vascular endothelial growth factor-induced signaling and angiogenesis**. Circ Res. 2002; 91: 1160-1167.

van Zandwijk N., Dalesio O., Pastorino U., de Vries N., van Tinteren H. **EUROSCAN, a randomized trial of vitamin A and N-acetylcysteine in patients with head and neck cancer or lung cancer. For the EUropean Organization for Research and Treatment of Cancer Head and Neck and Lung Cancer Cooperative Groups**. J Natl Cancer Inst. 2000; 92(12): 977-86.

Wiel C., Le Gal K., Ibrahim M.X., Jahangir C.A., Kashif M., Yao H., et al. **BACH1 Stabilization by Antioxidants Stimulates Lung Cancer Metastasis**. Cell. 2019; 178(2): 330-345.e22.

Yim C.Y., Hibbs Jr J.B.., McGregor J.R., Galinsky R.E., Samlowski W.E.. **Use of N-acetyl cysteine to increase intracellular glutathione during the induction of antitumor responses by IL-2**. J Immunol. 1994; 152(12): 5796–5805.

Yoo J., Hamilton S., Angel D., Fung K., Franklin J., Parnes L.S., et al. **Cisplatin otoprotection using transtympanic L-N-acetylcysteine: A pilot randomized study in head and neck cancer patients**. Laryngoscope. 2014; 124: E87–E94.

Zafarullah M., Li W.Q., Sylvester J., Ahmad M. **Molecular mechanisms of N-acetylcysteine actions**. Cell Mol Life Sci. 2003; 60: 6-20.

Zhang M., Hong X., Ma N., Wei Z., Ci X., Zhang S. **The promoting effect and mechanism of Nrf2 on cell metastasis in cervical cancer**. J Transl Med. 2023; 21(1):433.

Zhong H., Yin H. **Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: Focusing on mitochondria**. Redox Biol. 2015; 4: 193-199.

Review and Research on Cancer Treatment
Volume 9, Issue 1 (2023)

Genetically modified NK cells with enhanced reactivity – potential in treating therapy-resistant cancers

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ABSTRACT

Treatment-resistant cancers are a major challenge in clinical oncology, which requires the development of innovative treatment strategies. NK cells are a unique population of lymphoid cells with an innate ability to identify and eliminate virus-infected cells and cancer cells. The aim of this paper is to discuss the natural anticancer properties of NK cells, as well as the methods of their acquisition and modification aimed at enhancing anticancer activity. In particular, the mechanisms of cytotoxicity and the ability of NK cells to modulate the anti-tumour immune response were demonstrated. Problems related to the use of unmodified NK cells in cancer therapy are discussed. Methods of obtaining and modifying the anticancer activity of NK cells, including genetic modifications and metabolic reprogramming with the use of cytokines, were presented. Techniques such as chimeric antigen receptor (CAR) engineering and receptor-based modifications are discussed in detail. The possibilities of enhancing antitumor reactivity and NK cell persistence through costimulatory signalling, checkpoint inhibition and the use of cytokines are discussed. Preclinical and clinical studies demonstrating the efficacy of genetically engineered natural killer cells against a variety of treatment-resistant cancers have been reviewed with promising results. The development of allogeneic ready-to-use natural killer cell products has the potential to provide patients with immediate treatment options, bypassing the limitations of autologous cell therapies. Challenges and issues related to genetically modified NK cell therapies are discussed. Manufacturing scalability, potential side effects, and long-term safety concerns are important factors to consider during clinical translation. Ongoing research and clinical trials are discussed, highlighting the need for further validation and optimization of these therapies.

INTRODUCTION

Natural Killer cells play a crucial role within the innate immune system, serving as a frontline defence against both viral infections and malignant cells. Their unique ability to detect and eliminate aberrant cells without prior sensitization makes them highly attractive candidates for immunotherapy. However, despite their innate cytotoxic capabilities, NK (Natural Killer) cells encounter significant hurdles when targeting and eradicating therapy-resistant cancers. In recent years, the field of genetic engineering has emerged as a promising avenue to enhance NK cells reactivity and empower them to overcome these formidable barriers presented by resistant malignancies.

The aim of this work is to explore the intricacies of NK cell biology, uncovering their functions and mechanisms of action, to provide a detailed analysis of the multifaceted strategies used by cancer cells to develop resistance to therapies, shedding light on the intricate mechanisms at play, as well as to discuss the natural anticancer properties of NK cells. Additionally, we will explore the exciting field of genetic modifications that can significantly improve NK cell function and effectiveness against treatment-resistant cancers. In an era characterized by innovative breakthroughs in genetic engineering that are changing the landscape of cancer immunotherapy, this review aims to provide a comprehensive overview of the current state of NK cell-based therapies and the promising avenues they open in the ongoing battle against therapy-resistant cancers. By leveraging the powerful possibilities of genetic modification, we have the potential to revolutionize this field and instil newfound hope in patients facing enormous therapeutic challenges.

The work is of a review nature. The basis for assessing the current state of knowledge was a systematic review of the literature based on the following databases: PubMed, Science Direct and Wiley Online Library and other sources and materials related to the topic of the work in a direct or indirect way. In order to isolate all publications related to the topic of the work, selected sources were selected based on the use of keywords and keywords such as: "NK cells cancer", "NK cells cancer immunotherapy", "NK cells cancer therapy", "CAR–NK cells cancer", "NK cells cancer treatment". Based on this methodology, experimental and clinical studies were identified, the results of which were published in the years 1990-2023, in order to review and synthesize conclusions.

TREATMENR-RESISTANT TUMORS

Cancer treatment resistance remains a significant challenge in the field of oncology. Although the early successes of chemotherapy were promising, the emergence of drug resistance in cancer cells quickly overshadowed these achievements, leading to the adoption of combination chemotherapy (Vasan, 2019).

This approach has proven effective for some types of cancer, such as breast cancer and testicular cancer (Bonadonna, 1976; Bosl, 1986). However, it should be noted that the effectiveness of chemotherapy is limited in almost 90% of cases due to the development of drug resistance in cancer cells. This not only weakens the effectiveness of treatment, but also increases the aggressiveness of the cancer and facilitates its metastasis to other parts of the body (Vasan, 2019; Emran, 2022). In response to these challenges, new therapeutic strategies have emerged in oncology targeting fundamental cellular characteristics associated with carcinogenesis. These strategies include targeted therapies, which encompass a range of approaches including the use of tyrosine kinase inhibitors, nuclear receptor antagonists, and agents specifically designed to target and disrupt molecular pathways that promote cancer growth. Importantly, targeted therapies have shown significant effectiveness in certain types of cancer (Vasan, 2019). Another breakthrough in cancer treatment is immunotherapy. Monoclonal antibodies, such as those directed against immune checkpoints such as CTLA-4 (cytotoxic T cell antigen 4) and PD-1/PD-L1 (programmed death receptor 1/programmed death ligand 1), have revolutionized cancer therapy by blocking negative regulators or checkpoints within the immune system (Leach, 1996; Iwai, 2002). In some cases, these immunotherapies have achieved remarkable anti-tumor responses, giving patients new hope (Vasan, 2019). Additionally, new therapies such as dendritic cell therapy, CAR-T cell therapy, CAR-NK therapy, and many others have begun to achieve early successes (Neelapu, 2017; Liau, 2016; Liu, 2020). Despite these significant advances, it must be acknowledged that resistance remains a persistent challenge in the field of cancer therapy. Scientists and clinicians continue to explore innovative approaches to overcoming mechanisms of resistance and increasing the effectiveness of therapies, aiming to improve outcomes for people fighting cancer.

LIMITATIONS OF CONVENTIONAL THERAPIES AND MECHANISMS OF RESISTANCE

MDR (Multidrug Resistance) in cancer cells is a multifaceted phenomenon driven by a constellation of complex factors, each of which contributes to the resistance of these cells to chemotherapy. One of the hallmark aspects of MDR is the increased efflux of therapeutic agents through ABC transporters (ATP-binding cassette transporters), for example P-gp (P-glycoprotein) and BCRP (breast cancer resistance protein). These integral proteins exert significant control over the distribution, absorption, and elimination of a variety of chemicals, thereby protecting cells from the cytotoxic consequences of elevated intracellular drug concentrations. However, their presence may also interfere with drug delivery, leading to decreased bioavailability and decreased intracellular drug concentration (Mesci, 2019). Moreover, genetic mutations, widely considered a key cause of chemotherapy treatment failure, play a key role in this complex landscape. Mutations in key genes such as MYC, RAS and TP53 significantly influence the development of drug resistance (Duesberg, 2000). Furthermore, changes in the regulatory networks governed by miRNAs (microRNAs) and lncRNAs (long non-coding RNAs) further highlight the genetic complexity of MDR (Chen, 2016; Chen, 2019). Moreover, during carcinogenesis, the epigenome undergoes a series of profound changes, including global loss of DNA methylation, local hypermethylation, and pervasive changes in histone modification patterns. These epigenetic changes likely contribute significantly to the emergence of multidrug resistance (Kanwal, 2012). The tumor microenvironment also plays a key role in inducing drug resistance. In this complex environment, the antitumor functions of the immune system are disrupted, drug absorption is impaired, and cancer cell proliferation is promoted. Conditions such as hypoxia and autophagy in the tumor microenvironment further enhance drug resistance, significantly reducing the effectiveness of therapeutic interventions against cancer cells (Liang, 1996; van Vuuren, 2019). Moreover, additional factors, including selective therapeutic pressure that can lead to increased genomic instability and changes in tumor growth kinetics, as well as physical barriers, further contribute to the complex phenomenon of drug resistance (Sharma, 2019). This complex interplay of diverse factors highlights the powerful and challenging nature of drug resistance in the context of cancer.

BIOLOGY AND FUNCTIONS OF NK CELLS

NK cells are a population of cells characterized by a larger size compared to T and B lymphocytes and the presence of unique cytoplasmic granules (Chu, 2022). They originate from CD34+ hematopoietic stem cells located in the bone marrow and are widely distributed in various tissues, including the bloodstream, liver, and spleen (Yu, 2013). NK cells are part of the innate immune system and play a key role in monitoring and eliminating virus-transformed or infected cells; their effect is independent of previous immunization (Kwaśnik, 2020). Unlike T cells, they lack the TCR (T-cell receptor) and the associated CD3 complex responsible for signal transduction. NK cells are identified by the presence of the CD56

surface antigen and the lack of CD3 expression. They also express receptors such as CD16 and CD57 (Dalle, 2005). NK cells can be divided into two main subpopulations based on CD56 surface expression, each with distinct functions. CD56+ CD16-, characterized by high levels of CD56 and lack of CD16 expression, produce primarily cytokines. Conversely, CD56+CD16+ cells express moderate CD56 but high CD16 expression, resulting in greater cytotoxicity. Approximately 90% of NK cells in peripheral blood belong to the CD56+ CD16+ group (Kwasnik, 2020; Yu, 2013). NK cells have two primary functions: cytotoxicity and immune regulation. In the absence of prior activation, NK cells can identify and eliminate abnormal cells by releasing perforin and granzymes (Vivier, 2008). Stimulation of NK cells by KARs (killer activating receptors) triggers the expression and release of death ligands such as TNFα (tumour necrosis factor alpha), TRAIL (TNF-related apoptosis-inducing ligand), and FasL (Fas ligand), initiating the apoptosis pathway (Martínez- Lostao, 2015). Additionally, through their CD16 receptor, they can recognize cells coated with antibodies, initiating antibody-dependent cellular cytotoxicity (ADCC) and cytokine production (Kwaśnik, 2016). In their role as regulatory cells, NK cells produce a variety of cytokines and chemokines, including IL-10 (interleukin 10), IFN-γ (interferon-gamma), CCL3 (chemokine (CC motif) ligand 3), CCL4 (chemokine (CC motif)) ligand 4), CCL5 (chemokine (CC motif) ligand 3) and lymphotactin (Vivier, 2008). One study suggests that human NK cells may have memory-like properties. In support of this idea, studies have shown that when NK cells are initially activated with IL-12 (interleukin 12), IL-15 (interleukin 15), and IL-18 (interleukin 18), followed by a rest period of 1–3 weeks, they can induce a strong response characterized by increased production of IFN-γ upon subsequent exposure to cytokines or leukemic cells (Romme, 2012). This dual role of NK cells as both cytotoxic effectors and immune regulators highlights their importance in the body's defence mechanisms against cancer and infection.

THE ROLE OF NK CELLS IN THE ANTI-CANCER IMMUNE RESPONSE – MODIFYING IMPACT OF THE TUMOUR MICROENVIRONMENT

Anticancer mechanisms mediated by NK cells can be divided into direct and indirect actions. Direct mechanisms include several strategies used by NK cells to directly target cancer cells. First, they induce apoptosis in malignant cells by releasing perforin and granzyme upon direct contact. This approach is mainly used by cells with the CD56+CD16+ phenotype, effectively eliminating target cells, including those with reduced MHC (Major Histocompatibility Complex) class I expression (Chu, 2022). Second, NK cells with a CD56+CD16- phenotype initiate apoptosis without direct contact by binding membrane TNF family molecules to tumour cell ligands (Chu, 2022; Myers, 2021). Additionally, NK cells facilitate antibody-dependent cellular cytotoxicity (ADCC) (Hatjiharissi, 2007). Furthermore, NK cells produce cytokines, especially IFN-γ, which inhibit tumour angiogenesis and activate a specific immune response (Chu, 2022; Smyth, 2007). Turning to indirect mechanisms, NK cells possess immunomodulatory abilities by influencing various immune cells, including macrophages, T cells, and B cells, resulting in the production of numerous cytokines, growth factors, and chemokines (Chu, 2022). Upon activation, NK cells release IFN-γ, promoting the differentiation of CD8+ T cells into cytotoxic T cells and CD4+ T cells into Th1 (T helper 1) cells. Additionally, NK cells play a role in eliminating tumour cells and delivering tumour antigens to dendritic cells, causing dendritic cell maturation and antigen presentation (Nguyen-Pham, 2012). In the tumour microenvironment (TME), several mechanisms contribute to immunosuppression by hindering NK cell activity.

IMMUNOSUPRESIVE CYTOKINES IN TUMOUR MICROENVIRONMENT

An example is TGFβ (Transforming Growth Factor-beta), a strong immunosuppressive cytokine in the TME. TGFB inhibits NK cell function through mechanisms such as phosphorylation of Smad2/3 (maternal against decapentaplegic homolog 2/maternal against decapentaplegic homolog 3), limiting IFN-γ secretion, and influencing NK cell chemokine receptors (Du, 2021; Yu, 2006). TGFβ, mainly from tumour and regulatory T cells, downregulates NKG2D expression, inhibits the mTOR (mammalian target of rapamycin) pathway, and induces FBP1 (fructose-1,6-bisphosphatase) expression, collectively reducing NK cell activity. Additionally, cytokines such as IL-6 (interleukin 6) and potentially IL-8 (interleukin 8) contribute to impaired NK cell function by activating the STAT3 (signal transducer and activator of transcription 3) pathway (Wu, 2019).

IMMUNOSUPRESIVE METABOLIC COMPONENTS IN TUMOUR MICROENVIRONMENT

In the metabolic realm, cancer cells compete with activated NK cells for vital resources like glucose and glutamine, which are crucial for ATP (Adenosine triphosphate) generation and their rapid growth. Additionally metabolic factors such as indoleamine 2,3-dioxygenase, adenosine, and PGE2 (Prostaglandin E2) exert suppressive influences over NK cell proliferation and functional capacities (Du, 2021).

ABNORMAL LIGAND EXPPRESSION OF CANCER CELLS BOOSTS IMMUNE ESCAPE

A complex interaction involves the shedding of NKG2D ligands by cancer cells, facilitated by the ADAMs (disintegrin and metalloproteinase) family. This shedding leads to the proteolytic cleavage of MICA (MHC class I polypeptide–related sequence A), resulting in a reduction of MICA surface density (Waldhauer, 2008). Furthermore, cancer cells release soluble ligands like MICA/B, which bind to NKG2D. This binding hinders the interaction between NK cells and their target cells, subsequently downregulating NKG2D expression on the surface of NK cells, facilitating immune evasion (Du, 2021).

DYSFUNCTIONAL KREG CELLS REDUCE EFFECTOR CELL CYTOTOXICITY

In solid tumours, CD56bright NKreg (Natural Killer cell regulator) cells reshape the immunosuppressive microenvironment. They do this through high CD94-NKG2A expression and low CD16 expression, reducing cytotoxicity. These NKreg cells also secrete immunomodulatory factors, including IL-10 (Interleukin 10) and TGFβ. Moreover, their NKG2D and NKp46 (Natural Killer cell p46) receptors inhibit T cells proliferation and function (Fu, 2014).

INTERFERENCE BY OTHER IMMUNE CELLS IN TUMOUR MICROENVIRONMENT

Various immunosuppressive immune cells in the TME hinder NK cell activity. These include Tregs (regulatory T cells), MDSCs (myeloid-derived suppressor cells), TAMs (tumour-associated macrophages), and CAFs (tumour-associated fibroblasts). They use a variety of tactics, such as secreting immunosuppressive substances such as TGFβ and metabolites, disrupting interactions between NK cells and cancer cells through competition or decoy mechanisms, and releasing vesicles containing IL-37 (interleukin 37), which modifies NK cell function. Additionally, circulating platelet-coated tumour cells release TGFβ or express inhibitory receptor ligands, impeding NK cell activation and disrupting activating ligand expression (Placke, 2012; Chu, 2021; Vitale, 2014). Together, these mechanisms illustrate the complex interactions of factors in the TME that collectively contribute to suppressing NK cell activity in the context of cancer.

MECHANISMS OF REGULATION OF NK CELLS ACTIVITY

NK cells feature a balance of inhibitory and stimulatory receptors on their surface, crucial for regulating their immune responses. KIRs (Killer cell immunoglobulin-like receptors) are among these receptors, recognizing specific MHC class I alleles. NK cells also express NCRs (Natural cytotoxicity receptors) on their surface, facilitating cytotoxic mechanisms against cells with reduced or absent MHC class I expression. CD94/NKG2 lectin-like receptors constitute another essential group of NK cell receptors. CD94/NKG2 receptors detect non-classical HLA-E (Human Leukocyte Antigen E) class I molecules commonly found on cancer cells (Kwasnik, 2020; Borrego, 1998).

We can divide receptors into inhibitory and activating ones:

- inhibitory receptors include KIRs and C-type lectin receptors such as CD94/NKG2A/B. These receptors play a crucial role in maintaining NK cell quiescence (Chu, 2022);
- conversely, activating receptors play a pivotal role in stimulating NK cell responses and promoting their cytotoxic activities. They complement the inhibitory signals to ensure a balanced response. Activating receptors comprise cytotoxicity receptors (NKp44, NKp46, NKp30), C-type lectin receptors (NKG2E/H, CD94/NKG2C, NKG2F, NKG2D), and select KIRs (KIR-3DS and KIR-2DS) (Chu, 2022).

NK cells perform a complex assessment of the combination of these stimulatory and inhibitory signals to discern their target cells effectively. The ultimate outcome of NK cell activation depends on the unique attributes of the target cells, allowing NK cells to selectively eliminate aberrant cells while sparing healthy ones.

MODIFIED NK CELLS IN THE TREATMENT OF TREATMENT-RESISTANT CANCERS

NK cells have potential for clinical use despite resistance from cancer cells. In recent years, research in the field of NK cell-based cancer immunotherapy has flourished. Recent developments primarily focus on various strategies, including cytokine supplementation, monoclonal antibodies, modifications of the intrinsic signalling pathway, adoptive transfer, and genetic engineering of NK cells. These innovative approaches have opened up new opportunities in the quest to harness the full potential of NK cells in the fight against treatment-resistant cancers (Hodgins, 2019; Daher, 2018; Cheng 2013).

SOURCES AND METHODS OF OBTAINING NK CELLS

The use of NK cells in cancer therapy poses significant challenges due to their impaired functionality in cancer patients and limitations associated with autologous production (Platonova, 2011). To address these issues, allogeneic NK cells are favoured and can be derived from a variety of sources, including peripheral blood mononuclear cells, umbilical cord blood, immortalized cell lines, hematopoietic stem, and progenitor cells (HSPCs), and induced pluripotent stem cells (iPSCs). Each source has distinct advantages and disadvantages, influencing their genetic and functional characteristics (Laskowski, 2022). Primary NK cells can come from peripheral blood (PB-NK cells) or umbilical cord blood (CB-NK cells). CB-NK cells are readily available in blood banks, while PB-NK cells require donor-specific collection via apheresis (Laskowski, 2022; Dolstra 2017; Sharipo, 2022). Both sources have demonstrated successful applications in CAR (chimeric antigen receptor) redirected therapies. The selection of an appropriate NK cell source can be tailored to the specific requirements of patient populations and various diseases (Laskowski 2022). It is worth noting that donor-to-donor variability and interactions between HLA-KIR genotypes can significantly influence NK cell profiles and consequently influence clinical outcomes. The use of donor-derived NK cells may provide benefits, particularly when administered despite HLA-KIR genotype differences to counteract immune evasion by tumour cells (Ciurea, 2022). Selection of the NK cell source and consideration of genetic factors are critical aspects in the design of effective NK cell-based anticancer therapies.

METHODS OF ENHANCING THE ANTICANCER ACTIVITY OF NK CELLS

GENETIC MODIFICATIONS

Genetic manipulation of NK cells is extremely promising in the field of cancer immunotherapy, especially in the case of cancers that are resistant to destruction by NK cells (Kwaśnik, 2020). NK cell functionality is closely related to cytokines such as IL-2 (interleukin 2) and IL-15 (interleukin 15). However, systemic administration of these cytokines may induce an undesirable expansion of regulatory T cells. To circumvent these side effects, an interesting approach was used: direct introduction of the IL-2 gene into NK cells using various methods, using retroviruses and cDNA molecules (Kwasnik, 2020; Maddineni, 2022). This innovative strategy has yielded impressive results, especially when using NK-92 cells. The NK-92 cell line is characterized by abundant activating receptors but lacks some inhibitory receptors. This unique profile of NK-92 cells makes them suitable for clinical translation as they can be easily cultured *in vitro* (Ishikawa, 2018). These genetically modified NK-92 cells exhibit exceptional cytotoxicity and, even more remarkably, they possess the ability to effectively attack cancer cells regardless of specific tumour antigens. This has been convincingly demonstrated both in controlled laboratory conditions (*in vitro*) and in living organisms (*in vivo*) (Jochems, 2016; Tonn, 2013). Nevertheless, a limitation arises due to the inability of NK-92 cells to mediate antibody-dependent cellular cytotoxicity (ADCC). To address this limitation, Jochems and colleagues genetically modified NK-92 cells to introduce the high-affinity molecule CD16, a key factor in ADCC that is not normally found in NK-92 cells. In laboratory experiments, haNK (NK cells expressing a high-affinity Fc receptor) demonstrated potent cytotoxicity against various types of cancer, including lung and breast cancer. Moreover, the haNK system was further adapted to express a PD-L1-targeting CAR (Programmed Death-Ligand 1) called t-haNK, which showed promising efficacy both *in vitro* and *in vivo*, effectively controlling PD-L1-dependent tumours in mouse models (Klingemann, 2016; Jochems, 2016).

However, the use of NK-92 cell lines has specific limitations. First of all, these cell lines have limited expansion potential *in vivo*, which limits their ability to generate strong and durable effector responses. This limited expansion capacity ultimately reduces the maximum efficacy achievable with NK-92 cell therapies (Kang, 2021). Similar strategies that were used through IL-2 gene insertion were also used for the IL-15 gene, leading to increased NK cell proliferation and increased cytotoxicity (Kwasnik, 2020).

Another method of modification focuses on increasing the ability of NK cells to detect cancer cells by creating CARs (chimeric antigen receptors). CAR-developed T-cell therapies are at the forefront of immuno-oncology applications. While CAR-T cells can indeed achieve long-lasting responses due to their prolonged persistence and potential to differentiate into memory T-cell subsets, CAR-NK cells have the added advantage of being able to eliminate tumour cells regardless of CAR functionality. Additionally, CAR-T cells may be associated with significant associated toxicities, including cytokine release syndrome and neurotoxicity. In contrast, CAR-NK cells typically exhibit less toxicity, mainly due to their limited lifespan in circulation (Xie, 2020; Neelapu, 2017). CAR-NK cells are carefully designed to express receptors capable of recognizing specific tumour-associated antigens, resulting in increased cytotoxicity (Kwasnik, 2020). The evolution of CAR technology has led to various generations of CARs, including first-generation CARs with a basic structure and a single signalling region (Jensen, 2010); a second-generation CAR with an additional co-stimulatory domain such as CD28 (Maher, 2002); Third-generation CARs containing multiple co-stimulatory domains (Carpenito, 2009); and fourth-generation CARs combining multiple co-stimulatory domains with cytokine signals (Chu, 2022). CARs have been meticulously designed to target a wide range of antigens such as Her2/neu (erbB-2 receptor tyrosine-protein kinase/neurogenic differentiation factor 1), CD33, CD20, CD19, DAP12 (DNAX activating protein 12), FLT3 (FMS-Like Tyrosine Kinase 3) and/or CD33 (Kruschinski, 2009; Schirrmann, 2005; Muller, 2008; Imai, 2005; Töpfer, 2015; Tang, 2018). Notably, NK cells have inherent specificity and transient existence, making them an attractive option for generating CAR-positive cells (Maddineni, 2022). Studies have demonstrated the effectiveness and specificity of these cells in attacking cancer cells while maintaining a favourable safety profile, as seen in the treatment of CD19-positive lymphoid malignancies (Liu, 2022). The recent FDA approval of CD19-targeted CAR-T cell therapy represents a significant milestone in the development of CAR-positive cell therapies.

METABOLIC REPROGRAMMING USING CYTOKINES

Cytokines play a key role in supporting NK cell function and survival, especially IL-2 and IL-15, and this is important in the field of immunotherapy. These cytokines serve as potent stimulators of NK cell activity, but their systemic administration can sometimes lead to unintended side effects, such as the expansion of regulatory T cells, which can dampen the immune response (Du, 2022). To overcome these challenges, modified versions of these cytokines have been obtained. For example, a modified variant of IL-2 known as "super-2" has been developed that has increased binding affinity for IL-2Rβ (IL-2 receptor beta). This modification helps bypass problems associated with Treg cell interaction (Rosenberg, 1985; Levin, 2012, Du, 2022). Another innovative approach is to create an IL-2 fusion protein that selectively activates cells carrying NKG2D. This targeted activation promotes NK cell expansion without the adverse consequences associated with systemic IL-2 administration (Ghasemi, 2016). In the case of IL-15, early phase clinical trials investigated the use of rIL-15 (recombinant IL-15) in the treatment of refractory solid tumours. However, these studies have shown limited effectiveness, likely due to the inclusion of heavily pre-treated patients and the presence of less cytotoxic NK cells (Miller, 2018; Du, 2021). They also focused on obtaining hetIL-15 (heterodimeric IL-15), which offers extended half-lives and increased bioactivity. HetIL-15 has demonstrated the ability to promote greater persistence and expansion of NK cells and CD8+ T cells, which shows promise in the field of immunotherapy (Bergamaschi, 2021). Moreover, new approaches have emerged, such as N-803, an IL-15 superagonist. N-803 combines the IL-15 mutant with a fusion protein, significantly increasing its biological activity and extending its half-life (Rosario, 2016; Du, 2021). Clinical trials have provided evidence that N-803 when used in combination with immune checkpoint inhibitors or anti-CD20 monoclonal antibodies can increase NK cell cytotoxicity and lead to improved patient survival rates (Margolin, 2018). The goal of these innovative strategies is to unlock the full potential of cytokines while alleviating their limitations, ultimately increasing the effectiveness of NK cell-based therapy in cancer treatment.

BLOCKING KIR AND OTHER RECEPTORS USING MONOGLONAL ANTIBODIES

Inhibitory immunoglobulin-like receptors (KIRs) exert significant influence as potent regulators of NK cell activity, capable of bypassing concurrent activating signals upon interaction with HLA class I ligands. Due to their key role in suppressing NK cell function, inhibitory KIRs are of great interest scientists (Du, 2021). For example, IPH2101, an IgG monoclonal antibody directed against KIR2DL1/2/3 (killer immunoglobulin-like receptor domain long form 1/2/3), has undergone rigorous evaluation in clinical trials as

a stand-alone therapeutic agent. Although it was well tolerated, its ability to significantly increase anticancer potential was limited. Interestingly, its administration led to a reduction in the expression of inhibitory receptors KIR2D (Killer Immunoglobulin-like Receptor 2D) on NK cells (Carlsten, 2016). Of note, when IPH2101 was combined with Lenalidomide in people with relapsed/refractory multiple myeloma, a significant increase in median free survival was observed (Benson, 2015). On the other hand, another anti-KIR antibody, Lirilumab, failed to induce clinically significant responses in two early-stage clinical trials, thus halting its developmental progression (Vey, 2017; Vey, 2018). Additionally, the IPH4102 antibody demonstrated favourable tolerability in a Phase I clinical evaluation in people with relapsed/refractory cutaneous T-cell lymphoma (Bagot, 2019). The study of alternative inhibitory receptors such as CD96 and TIGIT (T cell immunoreceptor with immunoglobulin and ITIM domain) also shows great promise. These receptors are selectively expressed in human NK and T cells and recognize nectin and nectin-like ligands found in various types of cancer cells (Bottino, 2003; Martinet 2015). Notably, TIGIT expression is induced in NK cells upon activation, whereas CD96 is constitutively expressed. Both molecules show strong binding affinity to PVR (poliovirus receptor), although they have different effects on NK cell function. TIGIT interaction with PVR reduces NK cell cytotoxicity by activating DNAM-1 (DNAX-1 accessory molecule), while CD96 downregulates NK cell production of IFN-γ (Bernhardt, 2014; Chan, 2014). Fascinatingly, studies in melanoma and prostate cancer cell lines have provided evidence that administration of antibodies directed against CD96 increases the effectiveness of NK cells in fighting metastasis, primarily by hindering the CD96-CD155 interaction (Blake, 2016). Moreover, various immune checkpoints show potential in this context, including NKG2A and CD94 (Seymour, 2015). These multifaceted approaches highlight the complexity of modulating inhibitory receptors to enhance NK cell function in the field of cancer immunotherapy. Although some strategies show promise in clinical trials, others require further study and optimization.

USE OF BISPECIFIC AND MULTISPECIFIC ANTIBODIES

Bispecific antibodies (BsAbs) have received much attention, especially since the FDA approval of blinatumomab for the treatment of acute myeloid leukaemia (AML) (Labrjin, 2019). These antibodies are valued in the field of cancer therapy due to their characteristic ability to simultaneously bind two different epitopes (Viardot, 2018). Initially, the use of BsAbs was primarily oriented toward redirecting T cells toward tumour cells, an approach that emphasized enhancing the interaction between the extracellular CD3 subunit on T cells and tumour-associated antigens (Valdman, 2020). However, BsAb has also been studied in the context of NK cells. NK cells exhibit strong cytotoxicity, are less susceptible to exhaustion, and their cytotoxic activity does not involve binding to the MHC-Epitope complex (Ordóñez-Reyes, 2022). Work has begun on the development of NKCE (Natural Killer Cell Angers), a subclass of BsAb, with a modified Fc domain (fragment capable of crystallisation) to activate NK cells via NKp46. NKp46 plays a key role in inducing NK cell cytotoxicity, particularly in HLA class I unprotected cells, which is a common occurrence in cancers characterized by reduced HLA class I expression (Demaria, 2021). Growing evidence suggests that treatment with these antibodies can increase tumour infiltration by NK cells, consequently leading to strong anti-tumour responses in animal models (Gauthier, 2019).

Innovative NK cell strategies also include the development of NK cell engagers targeting CD16, in particular FcγRIIIA (Fc receptor gamma IIIA), the CD16A isoform. Currently, most NK cell engagement agents target antigens commonly expressed in hematologic malignancies, including CD19, CD20, CD30, and CD33. This trend is consistent with the trajectory of other immunotherapies such as CAR-T cells and CAR-NK cells (Ellwanger, 2019; Ordóñez-Reyes, 2022). As a pioneering FDA-approved bispecific antibody for the treatment of B-cell malignancies, blinatumomab represents a significant clinical milestone. However, many challenges remain, including issues related to treatment resistance and limited efficacy in solid tumours. In response to these challenges, significant efforts have been made to develop multispecific antibodies (Tapia-Galisteo, 2023). Multispecific antibodies are carefully designed to target activating NK cell receptors in combination with TAA (tumour-associated antigen), marking a promising trajectory in the cancer therapy landscape. The spectrum of NK cell receptor activation has been established in a hierarchical order based on their ability to activate quiescent NK cells, with CD16 > NKp46 > NKG2D dominating in potency (Bryceson, 2006). In the context of acute myeloid leukaemia, an antibody construct targeting CD33/CD16/CD123 was developed, resulting in enhanced NK cell-mediated lysis of primary leukemic cells compared to a trivalent bispecific antibody (Braciak, 2018). Moreover, other triumvirate antibody constructs have been developed targeting CD33 or HLA-DR (human leukocyte antigen-DR) and CD19.

These constructs engage NK cells via an anti-CD16 moiety or use the NKG2D ligand (Vyas, 2016). Additionally, a trispecific antibody format, called aTriFlex, was used to recalibrate NK cell cytotoxicity against two antigens found in multiple myeloma, significantly increasing its selectivity and efficacy (Gantke, 2018). In parallel to these multifaceted formats, IgG-like TsAbs (quadrivalent bispecific antibodies) have been carefully designed using various advanced technologies, including SEEDbody and common light chain technology. These TsAbs have the characteristic ability to simultaneously target multiple antigens such as EGFR (epidermal growth factor receptor), CD16, and PD-L1. This leads to increased binding affinity and increased potency of antibody-dependent cellular cytotoxicity (Pekar, 2020; Bogen, 2021).

Another notable strategy involves dual targeting of NK cell activation receptors. The ANKET (Antibody-based NK cell Engage Therapeutics) platform has achieved significant progress in this field, creating promising NKCEs targeting a diverse set of tumour antigens. These trifunctional NKCEs contain two Fab antibody fragments targeting NKp46 and TAA (CD19, CD20, EGFR), interspersed with an Fc domain to facilitate CD16-mediated ADCC. Preclinical studies have shown that these NKCEs demonstrate remarkable efficacy across a spectrum of cancer types, outperforming results obtained with monoclonal antibody treatment in a Raji B lymphoma model (Gauthier, 2019). Furthermore, in a study focusing on paediatric B-ALL (B-cell acute lymphoblastic leukaemia), trifunctional NKCEs targeting CD19 or CD20, engaging NKp46 or NKp30, demonstrated highly effective NK cell-mediated killing of leukaemia cell lines and primary blasts, in including resistant to the action of NK cells (Colomar-Carando, 2022). Additionally, the TriNKET (Triple-Negative Killer Engager Therapy) platform offers an alternative route to the development of multifunctional NKCEs. A notable agent within this platform, DF1001, is tailored to target HER2 while interacting with CD16 and NKG2D (Myers, 2021). The therapeutic potential of bispecific and multispecific antibodies, especially in the context of NK cell involvement, is a source of hope in the fight against cancer. As research and clinical trials progress, the continued search for multifaceted antibodies offers hope for better treatment outcomes for patients suffering from serious malignancies.

EFFICACY OF MODIFIED NK CELLS IN CANCER TREATMENT – CLINICAL TRIALS

NK cell therapy has shown promising anti-cancer effects in preclinical studies, targeting various malignancies like leukaemia, lymphoma, myeloma, ovarian cancer, and glioblastoma (Daher, 2021).

CAR-NK CELLS

When it comes to clinical trials, as of September 2023, there are more than 30 ongoing clinical trials investigating CAR-NK constructs for the treatment of various hematologic and solid tumour malignancies. These trials are registered on ClinicalTrials.gov. Approximately 70% of these trials are focused on haematological malignancies. Here are some noteworthy examples:

- immunotherapy combination: irradiated PD-L1 CAR-NK cells plus pembrolizumab plus N-803 for subjects with recurrent/metastatic gastric or head and neck cancer (NCT04847466);
- study of anti-PSMA CAR NK Cell (TABP EIC) in metastatic castration-resistant prostate cancer (NCT03692663);
- study of DLL3-CAR-NK cells in the treatment of extensive stage small cell lung cancer (NCT05507593);
- NKX019, intravenous allogeneic chimeric antigen receptor natural killer cells (CAR NK), in adults with B-cell cancers (NCT05020678);
- NKX101, intravenous allogeneic CAR NK cells, in adults with AML or MDS (NCT04623944).

These trials represent a promising avenue of research in the field of immunotherapy, where CAR-NK cells are being investigated for their potential to target and treat a range of cancers.

HERE ARE HIGHLIGHTED THE MOST IMPORTANT CLINICAL STUDIES ASSOCIATED WHITH THIS REVIEW

ACUTE MYELOID LEUKEMIA (AML)

In a significant development in 2018, the commencement of a first-in-human phase I clinical trial introduced CD33-CAR NK cells as a therapeutic modality for patients grappling with relapsed and refractory AML. This pioneering study, which enrolled a trifecta of participants, placed paramount emphasis on evaluating the safety profile of CD33-CAR-NK cells. Notably, the investigation yielded a noteworthy absence of reported adverse events (Tang, 2018; Chu, 2022).

MULTIPLE MYELOMA (MM)

As previously highlighted, a noteworthy clinical trial phase involving IPH2101, when combined with lenalidomide in a cohort of fifteen individuals afflicted by relapsed/refractory multiple myeloma, demonstrated a substantial enhancement in median progression-free survival (Benson, 2015). Additionally, an independent exploration of IPH2101 as a monotherapy among a group of nine patients revealed overall tolerability but limited efficacy in significantly enhancing anti-cancer potential (Carlsten, 2016).

LYMPHOMA

In 2020, a remarkable milestone was achieved in the treatment of high-risk B-cell lymphoma and CD19+ chronic lymphocytic leukaemia (CLL) through the use of allogeneic cord blood-derived CAR-NK cells. This approach was characterized by a favourable safety profile, in particular it did not cause side effects such as cytokine release syndrome, neurotoxicity or GVHD (graft-versus-host disease). Impressively, 73% of patients responded positively to this intervention, achieving complete or partial remission. These responses were rapid and lasted for at least 30 days after infusion (Liu, 2020). Additionally, Bachanova and colleagues presented preliminary clinical results with FT596, an off-the-shelf induced pluripotent stem cell (iPSC)-derived CAR-NK therapy targeting CD19 in the context of B-cell lymphoma. Preliminary observations on the effects of FT596 treatment in one patient indicated a partial response characterized by with a reduction in tumour volume exceeding 50%. However, it is worth noting that several adverse events have been reported, including leukopenia, neutropenia, anaemia, and urinary tract problems (Bachanova, 2020).

SOLID TUMOURS

In the Phase I dose escalation study, the primary objectives were to evaluate the feasibility of large-scale expansion and to assess the safety of administering ex vivo-expanded NK-92 cells as allogeneic cellular immunotherapy to patients with refractory cancer, renal cell carcinoma and melanoma. This study included a cohort of twelve patients, and infusion toxicities associated with NK-92 cell administration were mainly mild. Interestingly, one patient demonstrated durable disease survival four years after NK-92 infusion. Additionally, among the patients, one person with metastatic melanoma showed a modest response during the study period, while another patient had a mixed response (Arai, 2008). Additionally, in a clinical trial, a cohort of fifteen people diagnosed with advanced and refractory cancers, mainly solid tumours and sarcomas, and in some cases leukaemia or lymphoma, received two doses of NK-92 cells given 48 hours apart. Impressively, no adverse events were reported during or after the infusion. Notably, 75% of lung cancer patients showed some form of positive response (Tonn, 2013). In recent developments, ongoing clinical trials are aimed at evaluating the PD-L1 t-haNK system. These cells, derived from NantKwest's proprietary master NK-92 (aNK) cell bank, are genetically engineered to target PD-L1 and produce intracellular IL-2, thereby enhancing their capacity for antibody-targeted cellular cytotoxicity. CD16. Preliminary observations from this study, pending peer review, showed no dose-limiting toxicities in a cohort of six patients diagnosed with locally advanced or metastatic solid tumours. This trial is registered under the identifier (ClinicalTrials.gov: NCT04050709). As previously mentioned, a Phase I clinical trial provided evidence of the utility of recombinant rIL-15 in the treatment of refractory solid tumours in 19 patients. Although no objective responses were observed, several patients experienced disease stabilization (Miller, 2018). Additionally, as previously highlighted, a Phase I clinical trial involving a cohort of 24 patients provided evidence that the use of ALT-803 in combination with immune checkpoint inhibitors or anti-CD20 monoclonal antibodies may increase NK cell cytotoxicity and contribute to better patient survival rates (Margolin, 2018).

CHALLENGES ASSOCIATED WITH MODIFIED NK CELL-BASED THERAPIES

NK cell-based therapy faces several significant challenges. First, ex vivo NK cell expansion represents a significant hurdle, primarily attributed to the inherent difficulties in obtaining sufficient NK cells from a single donor (Chu, 2022). Second, the crucial task of selecting the most appropriate CAR transduction method into NK cells cannot be underestimated. Although viral vectors such as retroviruses are commonly used, their use is associated with potential risks, including insertional mutations and carcinogenesis (Myers, 2021). Third, the complex and multifaceted tumour microenvironment introduces a multitude of complexities into the field of NK therapy (Daher, 2021). Finally, evaluation of preclinical outcomes faces significant obstacles due to the lack of clinically relevant animal models that can faithfully reproduce the intricacies

and interactions in the TME. Unfortunately, the vast majority of studies continue to rely on immunocompromised mice, which, while valuable, do not sufficiently mimic the clinically relevant TME and do not provide a precise assessment of NK cell functionality (Chu, 2022). To optimize NK cell therapeutic strategies, it becomes necessary to obtain a comprehensive understanding of the impact of metabolic markers and signalling pathways on NK cell persistence. Additionally, it is critical to refine manufacturing procedures to ensure consistency and uniformity in NK cell infusions. It is equally important to explore strategies to preserve NK cell persistence without inducing lymphodepletion, especially in the context of co-administration with chemotherapy or radiotherapy (Du, 2021).

DIRECTIONS FOR FURTHER RESEARCH ON THE USE OF MODIFIED NK CELLS IN CANCER THERAPY

The landscape of clinical trials pertaining to NK cell-based immunotherapy encompasses phases I and II, with a predominant focus on haematological malignancies (Wu, 2020). However, NK cells have consistently demonstrated remarkable safety and efficacy profiles in these clinical trials. Whether administered as standalone interventions or in conjunction with other therapeutic modalities, both allogeneic and autologous approaches have exhibited promising outcomes (Laskowski, 2022). Of particular significance is the application of chimeric antigen receptor (CAR)-engineered NK cells, which have proven to be highly effective in targeting tumour-specific antigens and thereby amplifying anti-tumour responses (Zhang, 2022).

Moreover, the development of antibodies meticulously tailored to selectively target inhibitory receptors on NK cells, encompassing KIRs, NKG2A, and TIGIT, has unveiled their potential in enhancing NK cell responses and fortifying the elimination of tumour cells. Several of these antibody candidates are currently undergoing rigorous clinical validation (Du, 2021). Capitalizing on the expansive recognition capabilities intrinsic to NK cells, the integration of NK cell-based therapies into multifaceted immune combination strategies holds substantial promise for further augmenting anti-tumour efficacy. Prospective innovations may involve more intricate genetic modifications aimed at bolstering the longevity and functionality of NK cells while mitigating the risk of unintended side effects (Maddineni, 2022). A forthcoming challenge in the adoption of NK cell-based therapy lies in the need for a more precise characterization of distinct NK cell subsets. This necessitates the identification of specific markers, functional attributes, and regulatory pathways unique to each subgroup. Such insights will facilitate the development of tailored therapeutic approaches for the treatment of tumours that are infiltrated by distinct NK cell populations. Furthermore, to mitigate off-target effects induced by conventional anti-tumour medications within the tumour microenvironment, future clinical trials should incorporate meticulously selected combination therapies. This strategic refinement is pivotal to ensure that treatment effectiveness remains uncompromised (Wu, 2020).

Delving into other subsets of ILCs (Innate Lymphoid Cells) beyond NK cells, such as intraepithelial ILC1-like cells, holds the potential to bolster antitumor responses and broaden the horizons of NK cell therapies in the realm of cancer immunotherapy (Moreno-Nieves, 2021).

CONCLUSIONS

The development and application of genetically modified NK cells with enhanced reactivity hold significant promise in the treatment of therapy-resistant cancers. NK cells exhibit a remarkable ability to target and eliminate cancer cells through various mechanisms, both directly and indirectly. Genetic modifications, such as CAR expression and cytokine supplementation, have shown immense potential in augmenting NK cell cytotoxicity and persistence within the tumour microenvironment. Moreover, the use of monoclonal antibodies targeting inhibitory receptors on NK cells, along with advancements in allogeneic NK cell therapies, offers novel approaches to overcoming immune evasion strategies employed by tumour cells. These strategies broaden the horizons of cancer immunotherapy and hold the potential to improve patient outcomes, particularly in cases where conventional treatments have failed.

While challenges persist, such as optimizing expansion protocols, selecting appropriate sources for NK cells, and improving our understanding of the intricate interactions within the tumour microenvironment, ongoing research and innovation continue to drive the field forward. As we delve into the realm of emerging NK cell subtypes and their potential synergies with other immune therapies, the future of genetically modified NK cell-based treatments for therapy-resistant cancers appears promising.

References

Arai S., Meagher R., Swearingen M., Myint H., Rich E., Martinson J., et al. **Infusion of the allogeneic cell line NK-92 in patients with advanced renal cell cancer or melanoma: a phase I trial**. Cytotherapy. 2010; 10(6): 625-632.

Bachanova V., Cayci Z., Lewis D., Maakaron J.E., Janakiram M., Bartz A., et al. **Clinical activity of FT596, a first-in-class, Multi-Antigen targeted, off-the-shelf, iPSCderived CD19 CAR NK cell therapy in relapsed/refractory B-cell lymphoma**. Blood. 2020; 136: 8-8.

Bagot M., Porcu P., Marie-Cardine A., Battistella M., William B.M., Vermeer M., et al. I**PH4102, a first-in-class anti-KIR3DL2 monoclonal antibody, in patients with relapsed or refractory cutaneous T-cell lymphoma: an international, first-in-human, open-label, phase 1 trial**. Lancet Oncol. 2019; 20(8): 1160-1170.

Bergamaschi C., Stravokefalou V., Stellas D., Karaliota S., Felber, B.K., Pavlakis G.N. **Heterodimeric IL-15 in Cancer Immunotherapy**. Cancers. 2021; 13(4): 837.

Bernhardt G. **TACTILE becomes tangible: CD96 discloses its inhibitory peculiarities**. Nat Immunol. 2014; 15(5): 406-408.

Benson D.M., Cohen A.D., Jagannath S., Munsbrhi N.C., Spitzer G., Hofmeister C.C., et al. **A Phase I Trial of the Anti-KIR Antibody IPH2101 and Lenalidomide in Patients with Relapsed/Refractory Multiple Myeloma. Clinical cancer research.** Clin Cancer Res. 2015; 21(18): 4055-4061.

Blake S.J., Stannard K., Liu J., Allen S., Yong M.C., Mittal D., et al. **Suppression of metastases using a new lymphocyte checkpoint target for cancer immunotherapy**. CancerDiscov. 2016; 6(4): 446-59.

Bogen J.P., Carrara S.C., Fiebig D., Grzeschik J., Hock B., Kolmar H. **Design of a Trispecific Checkpoint Inhibitor and Natural Killer Cell Engager Based on a 2 + 1 Common Light Chain Antibody Architecture**. Frontiers immunol. 2021; 12: 669496.

Bonadonna G., Brusamolino E., Valagussa P., Rossi A., Brugnatelli L., Brambilla C., et al. **Combination chemotherapy as an adjuvant treatment in operable breast cancer**. N Engl J Med. 1976; 294(8): 405-410.

Borrego F., Ulbrecht M., Weiss E.H., Coligan J.E., Brooks A.G. **Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis**. J Exp Med. 1998; 187(5): 813-818.

Bosl G.J., Gluckman R., Geller N.L., Golbey R.B., Whitmore W.F., Jr. Herr H., et al. **VAB-6: an effective chemotherapy regimen for patients with germ-cell tumors**. J Clin Oncol. 1986; 4(10): 1493-1499.

Bottino C., Castriconi R., Pende D., Rivera P., Nanni M., Carnemolla B., et al. **Identification of PVR (CD155) and Nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule**. J Exp Med. 2003; 198(4): 557-567.

Braciak T.A., Roskopf C.C., Wildenhain S., Fenn N.C., Schiller C.B., Schubert I.A., et al. **Dual-targeting triplebody 33-16-123 (SPM-2) mediates effective redirected lysis of primary blasts from patients with a broad range of AML subtypes in combination with natural killer cells**. Oncoimmunology. 2018; 7(9): e1472195.

Brand A., Singer K., Koehl G.E., Kolitzus, M., Schoenhammer G., Thiel A., et al. **LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells**. Cell Metab. 2016; 24(5): 657-671.

Bryceson Y.T., March M.E., Ljunggren H.G., Long E.O. **Synergy among receptors on resting NK cells for the activation of natural cytotoxicity and cytokine secretion**. Blood. 2006; 107: 159-66.

Carlsten M., Korde N., Kotecha R., Reger R., Bor S., Kazandjian D., et al. **Checkpoint Inhibition of KIR2D with the Monoclonal Antibody IPH2101 Induces Contraction and Hyporesponsiveness of NK Cells in Patients with Myeloma**. Clin Cancer Res. 2016; 22(21): 5211-5222.

Carpenito C., Milone M.C., Hassan R., Simonet J.C., Lakhal M., Suhoski M.M., et al. **Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains**. Proc Natl Acad Sci U S A. 2009; 106(9): 3360-3365.

Chan C.J., Martinet L., Gilfillan S., Souza-Fonseca-Guimaraes F., Chow M.T., Town L., et al. **The receptors CD96 and CD226 oppose each other in the regulation of natural killer cell functions**. Nat Immunol. 2014; 15(5): 431-438.

Chen X., Lu P., Wang D.D., Yang S.J., Wu Y., Shen H.Y., et al. **The role of miRNAs in drug resistance and prognosis of breast cancer formalin-fixed paraffin-embedded tissues**. Gene. 2016; 595(2): 221-226.

Chen Y., Liu L., Li J., Du Y., Wang J., Liu J. **Effects of long noncoding RNA (linc-VLDLR) existing in extracellular vesicles on the occurrence and multidrug resistance of esophageal cancer cells**. Pathol Res Pract. 2019; 215(3): 470-477.

Cheng M., Chen Y., Xiao W., Sun R., Tian Z. **NK cell-based immunotherapy for malignant diseases**. Cell Mol Immunol. 2013; 10(3): 230-252.

Cho B.C., Abreu D.R., Hussein M., Cobo M., Patel A. J., Secen N., et al. **Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study**. Lancet. Oncol. 2022; 23(6): 781-792.

Chu J., Gao F., Yan M., Zhao S., Yan Z., Shi B., et al. **Natural killer cells: a promising immunotherapy for cancer**. J Transl Med. 2022; 20(1): 240.

Ciurea S.O., Kongtim P., Soebbing D., Trikha P., Behbehani G., Rondon G., et al. **Decrease post-transplant relapse using donor-derived expanded NK-cells**. Leukemia. 2022; 36(1): 155-164.

Colomar-Carando N., Gauthier L., Merli P., Loiacono F., Canevali P., Falco M., et al. **Exploiting Natural Killer Cell Engagers to Control Pediatric B-cell Precursor Acute Lymphoblastic Leukemia**. Cancer Immunol Res**.** 2022; 10(3): 291-302.

Cong J., Wang X., Zheng X., Wang D., Fu B., Sun R., et al. **Dysfunction of Natural Killer Cells by FBP1-Induced Inhibition of Glycolysis during Lung Cancer Progression**. Cell Metab. 2018; 28(2): 243-255.

Dalle J.H., Menezes J., Wagner E., Blagdon M., Champagne J., Champagne M.A., et al. **Characterization of Cord Blood Natural Killer Cells: Implications for Transplantation and Neonatal Infections**. Pediatr Res. 2005; 57: 649-655.

Daher M., Rezvani K. **Next generation natural killer cells for cancer immunotherapy: the promise of genetic engineering**. Curr Opin Immunol. 2018; 51: 146-153.

Daher M., Rezvani K. **Outlook for New CAR-Based Therapies with a Focus on CAR NK Cells: What Lies Beyond CAR-Engineered T Cells in the Race against Cancer**. Cancer Discov. 2021; 11(1): 45-58.

Demaria O., Gauthier L., Debroas G., Vivier E. **Natural killer cell engagers in cancer immunotherapy: Next generation of immuno-oncology treatments**. Eur J Immunol. 2021; 51(8): 1934-1942.

Dolstra H., Roeven M.W.H., Spanholtz J., Hangalapura B.N., Tordoir M., Maas F., et al. **Successful Transfer of Umbilical Cord Blood CD34+ Hematopoietic Stem and Progenitor-derived NK Cells in Older Acute Myeloid Leukemia Patients. Clinical cancer research**. Clin Cancer Res. 2016; 23(15): 4107-4118.

Du N., Guo F., Wang Y., Cui J. **NK Cell Therapy: A Rising Star in Cancer Treatment**. Cancers (Basel). 2021; 13(16): 4129.

Duesberg P., Stindl R., Hehlmann R. **Explaining the high mutation rates of cancer cells to drug and multidrug resistance by chromosome reassortments that are catalyzed by aneuploidy**. Proc Natl Acad Sci U S A. 2000; 97(26): 14295-14300.

Ellwanger K., Reusch U., Fucek I., Wingert S., Ross T., Müller T., et al. **Redirected optimized cell killing (ROCK®): A highly versatile multispecific fit-for-purpose antibody platform for engaging innate immunity**. MAbs. 2019; 11(5): 899-918.

Elshiaty M., Schindler H., Christopoulos P. **Principles and Current Clinical Landscape of Multispecific Antibodies against Cancer**. Int J Mol Sci. 2021; 22(11): 5632.

Emran T.B., Shahriar A., Mahmud A.R., Rahman T., Abir M.H., Siddiquee M.F., et al. **Multidrug Resistance in Cancer: Understanding Molecular Mechanisms, Immunoprevention and Therapeutic Approaches**. Front Oncol. 2022; 12: 891652.

Fu B., Tian Z., Wei H. **Subsets of human natural killer cells and their regulatory effects**. Immunology. 2014; 141(4): 483-489.

Gantke T., Weichel M., Herbrecht C., Reusch U., Ellwanger K., Fucek I., et al. **Trispecific antibodies for CD16A-directed NK cell engagement and dual-targeting of tumor cells. Protein engineering, design & selection**. Protein Eng Des Sel. 2017; 30(9): 673-684.

Gauthier L., Morel A., Anceriz N., Rossi B., Blanchard-Alvarez A., Grondin G., et al. **Multifunctional Natural Killer Cell Engagers Targeting NKp46 Trigger Protective Tumor Immunity**. Cell. 2019; 177(7): 1701-1713.

Ghasemi R., Lazear E., Wang X., Arefanian S., Zheleznyak A., Carreno B.M., et al. **Selective targeting of IL-2 to NKG2D bearing cells for improved immunotherapy**. Nat Commun. 2016; 7: 12878.

Hatjiharissi E., Xu L., Santos D.D., Hunter Z.R., Ciccarelli B.T., Verselis S., et al. **Increased natural killer cell expression of CD16, augmented binding and ADCC activity to rituximab among individuals expressing the Fc{gamma}RIIIa-158 V/V and V/F polymorphism**. Blood. 2007; 110(7): 2561-2564.

Hodgins J.J., Khan S.T., Park M.M., Auer R.C., Ardolino M. **Killers 2.0: NK cell therapies at the forefront of cancer control**. J Clin Invest. 2019; 129(9): 3499-3510.

Imai C., Iwamoto S., Campana D. **Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells**. Blood. 2005; 106(1): 376-83.

Ishikawa T., Okayama T., Sakamoto N., Ideno M., Oka K., Enoki T., et al. **Phase I clinical trial of adoptive transfer of expanded natural killer cells in combination with IgG1 antibody in patients with gastric or colorectal cancer**. Int J Cancer. 2018; 142 :2599-609.

Iwai Y., Ishida M., Tanaka Y., Okazaki T., Honjo T., Minato N. **Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade**. Proc Natl Acad Sci U S A. 2002; 99(19): 12293-12297.

Jensen M.C., Popplewell L., Cooper L.J., DiGiusto D., Kalos M., Ostberg J.R., et al. **Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. Biology of blood and marrow transplantation**. Biol Blood Marrow Transplant. 2010; 16(9): 1245-1256.

Jochems C., Hodge J.W., Fantini M., Fujii R., Morillon Y.M., Greiner J.W., et al. **An NK cell line (haNK) expressing high levels of granzyme and engineered to express the high affinity CD16 allele**. Oncotarget. 2016; 7(52): 86359-86373.

Kang S., Gao X., Zhang L., Yang E., Li Y., Yu L. **The advances and challenges of NK cell-based cancer immunotherapy**. Curr Oncol. 2021; 28: 1077-93.

Kanwal R., Gupta S. **Epigenetic modifications in cancer**. Clin Genet. 2012; 81: 303-311.

Khan M., Arooj S., Wang H. **NK Cell-Based Immune Checkpoint Inhibition**. Front Immunol. 2020; 11: 167.

Klingemann H., Boissel L., Toneguzzo F. **Natural Killer Cells for Immunotherapy - Advantages of the NK-92 Cell Line over Blood NK Cells**. Front Immunol. 2016; 7: 91.

Kruschinski A., Moosmann A., Poschke I., Norell H., Chmielewski M., Seliger B., et al. **Engineering antigen-specific primary human NK cells against HER-2 positive carcinomas**. Proc Natl Acad Sci U S A. 2008; 105(45): 17481-17486.

Kwaśnik P., Lemieszek M.K., Rzeski W. **Possibilities of using NK cells in cancer immunotherapy**. Med Og Nauk Zdr. 2020; 26(1): 8-16.

Labrijn A., Janmaat M., Reichert J., Parren P. **Bispecific Antibodies: A Mechanistic Review of the Pipeline**. Nat. Rev. Drug Discov. 2019; 18: 585-608.

Laskowski T.J., Biederstädt A., Rezvani K. **Natural killer cells in antitumour adoptive cell immunotherapy**. Nat Rev Cancer. 2022; 22: 557-575.

Leach D.R., Krummel M.F., Allison J.P. **Enhancement of antitumor immunity by CTLA-4 blockade**. Science. 1996; 271(5256): 1734-1736.

Levin A.M., Bates D.L., Ring A.M., Krieg C., Lin J.T., Su L., et al. **Exploiting a natural conformational switch to engineer an interleukin-2 'superkine'**. Nature. 2012; 484(7395): 529-533.

Liang B.C. **Effects of hypoxia on drug resistance phenotype and genotype in human glioma cell lines**. J Neurooncol. 1996; 29(2): 149-155.

Liau L.M., Ashkan K., Tran D.D., Campian J.L., Trusheim J. E., Cobbs C.S., et al. **First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma**. J Transl Med. 2018; 16(1): 142.

Liu E., Marin D., Banerjee P., Macapinlac H.A., Thompson P., Basar R., et al. **Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors**. N Engl J Med. 2022; 382(6): 545-553.

Maddineni S., Silberstein J.L., Sunwoo J.B. **Emerging NK cell therapies for cancer and the promise of next generation engineering of iPSC-derived NK cells.** J Immunother Cancer**.** 2022; 10(5): e004693.

Maher J., Brentjens R.J., Gunset G., Rivière I., Sadelain M. **Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta /CD28 receptor**. Nat Biotechnol. 2002; 20(1): 70-75.

Margolin K., Morishima C., Velcheti V., Miller J.S., Lee S.M., Silk A., et al. **Phase I Trial of ALT-803, A Novel Recombinant IL15 Complex, in Patients with Advanced Solid Tumors**. Clin Cancer Res. 2018; 24(22): 5552–5561.

Martinet L., Smyth MJ. **Balancing natural killer cell activation through paired receptors**. Nat Rev Immunol. 2015; 15(4): 243-254.

Martínez-Lostao L., Anel A., Pardo J. **How Do Cytotoxic Lymphocytes Kill Cancer Cells?** Clin Cancer Res. 2015; 21(22): 5047-5056.

Mesci S., Marakli S., Yazgan B., Yıldırım T. **The effect of ATP-binding cassette (ABC) transporters in human cancers**. J Evid Based Med. 2019; 1(1): 14-19.

Miller J.S., Morishima C., McNeel D.G., Patel M.R., Kohrt H.E.K., Thompson J. A., et al. **A First-in-Human Phase I Study of Subcutaneous Outpatient Recombinant Human IL15 (rhIL15) in Adults with Advanced Solid Tumors**. Clin Cancer Res. 2018; 24(7): 1525-1535.

Moreno-Nieves U.Y., Tay J.K., Saumyaa S., Horowitz N.B., Shin J.H., Mohammad I.A., et al. **Landscape of innate lymphoid cells in human head and neck cancer reveals divergent NK cell states in the tumor microenvironment**. Proc Natl Acad Sci U S A. 2021; 118(28): e2101169118.

Müller T., Uherek C., Maki G., Chow K.U., Schimpf A., Klingemann H.G., et al. **Expression of a CD20-specific chimeric antigen receptor enhances cytotoxic activity of NK cells and overcomes NK-resistance of lymphoma and leukemia cells**. Cancer Immunol Immunother. 2008; 57(3): 411-423.

Myers J.A., Miller J.S. **Exploring the NK cell platform for cancer immunotherapy**. Nat Rev Clin Oncol. 2021; 18: 85-100.

Neelapu S.S., Locke F.L., Bartlet, N.L., Lekakis L.J., Miklos D.B., Jacobson C.A., et al. **Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma**. N Engl J Med. 2017; 377(26): 2531-2544.

Nguyen-Pham T.N., Yang D.H., Nguyen T.A., Lim M.S., Hong C.Y., Kim M.H., et al. **Optimal culture conditions for the generation of natural killer cell-induced dendritic cells for cancer immunotherapy**. Cell Mol Immunol. 2012; 9(1): 45-53.

Nisonoff A., Rivers M.M. **Recombination of a mixture of univalent antibody fragments of different specificity**. Arch Biochem Biophys.1961; 93: 460-2.

Ordóñez-Reyes C., Garcia-Robledo J.E., Chamorro D.F., Mosquera A., Sussmann L., Ruiz-Patiño A., et al. **Bispecific Antibodies in Cancer Immunotherapy**. Pharmaceutics. 2022; 14(6): 1243.

Pekar L., Busch M., Valldorf B., Hinz S.C., Toleikis L., Krah S. **Biophysical and biochemical characterization of a VHH-based IgG-like bi- and trispecific antibody platform**. MAbs. 2020; 12(1): 1812210.

Placke T., Salih H.R., Kopp H.G. **GITR ligand provided by thrombopoietic cells inhibits NK cell antitumor activity**. J Immunol. 2012; 189(1): 154-160.

Platonova S., Cherfils-Vicini J., Damotte D., Crozet L., Vieillard V., Validire P., et al. **Profound coordinated alterations of intratumoral NK cell phenotype and function in lung carcinoma**. Cancer Res. 2011; 71(16): 5412-5422.

Romee R., Schneider S.E., Leong J.W., Chase J.M., Keppel C.R., Sullivan R.P., et al. **Cytokine activation induces human memory-like NK cells**. Blood. 2012; 120(24): 4751-4760.

Rosario M., Liu B., Kong L., Collins L.I., Schneider S.E., Chen X., et al. **The IL-15-Based ALT-803 Complex Enhances FcγRIIIa-Triggered NK Cell Responses and In Vivo Clearance of B Cell Lymphomas**. Clin Cancer Res. 2016; 22(3): 596-608.

Rosenberg S.A., Lotze M.T., Muul L.M., Leitman S., Chang A.E., Ettinghausen S.E., et al. **Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer**. N Engl J Med. 1985; 313(23): 1485-1492.

Seymour L., Tinker A., Hirte H., Wagtmann N., Dodion P. **Phase I and dose ranging, phase II studies with IPH2201, a humanized monoclonal antibody targeting HLA-E receptor CD94/NKG2A**. Ann Oncol. 2015; 26: ii3.

Schirrmann T., Pecher G. **Specific targeting of CD33+ leukemia cells by a natural killer cell line modified with a chimeric receptor**. Leuk Res. 2005; 29(3): 301-6.

Shapiro R.M., Birch G.C., Hu G., Vergara Cadavid J., Nikiforow S., Baginska J., et al. **Expansion, persistence, and efficacy of donor memory-like NK cells infused for posttransplant relapse**. J Clin Invest. 2022; 132(11): e154334.

Sharma P., Hu-Lieskovan S., Wargo J.A., Ribas A. **Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy**. Cell. 2017; 168(4): 707-723.

Smyth M.J., Crowe N.Y., Pellicci D.G., Kyparissoudis K., Kelly J.M., Takeda K., et al. **Sequential production of interferon-gamma by NK1.1(+) T cells and natural killer cells is essential for the antimetastatic effect of alpha-galactosylceramide**. Blood. 2002; 99(4): 1259-1266.

Tang X., Yang L., Li Z., Nalin A.P., Dai H., Xu T., et al. **First-in-man clinical trial of CAR NK-92 cells: safety test of CD33-CAR NK-92 cells in patients with relapsed and refractory acute myeloid leukemia**. Am J Cancer Res. 2018; 8(6): 1083-1089.

Tapia-Galisteo A., Compte M., Álvarez-Vallina L., Sanz, L. **When three is not a crowd: trispecific antibodies for enhanced cancer immunotherapy**. Theranostics. 2023; 13(3): 1028-1041.

Tonn T., Schwabe D., Klingemann H.G., Becker S., Esser R., Koehl U., et al. **Treatment of patients with advanced cancer with the natural killer cell line NK-92**. Cytotherapy. 2013; 15(12): 1563-1570.

Töpfer K., Cartellieri M., Michen S., Wiedemuth R., Müller N., Lindemann D., et al. **DAP12-based activating chimeric antigen receptor for NK cell tumor immunotherapy**. J Immunol. 2015; 194(7): 3201-3212.

van Vuuren R.J., Botes M., Jurgens T., Joubert A.M., van den Bout I. **Novel sulphamoylated 2-methoxy estradiol derivatives inhibit breast cancer migration by disrupting microtubule turnover and organization**. Cancer Cell Int. 2019; 19: 1.

Vasan N., Baselga J., Hyman D.M. **A view on drug resistance in cancer.** Nature. 2019; 575(7782): 299-309.

Vey N., Karlin L., Sadot-Lebouvier S., Broussais F., Berton-Rigaud D., Rey J., et al. **A phase 1 study of lirilumab (antibody against killer immunoglobulin-like receptor antibody KIR2D; IPH2102) in patients with solid tumors and hematologic malignancies**. Oncotarget. 2018; 9(25): 17675-17688.

Viardot A., Bargou R. **Bispecific Antibodies in Haematological Malignancies**. Cancer Treat. Rev. 2018; 65: 87-95.

Vitale M., Cantoni C., Pietra G., Mingari M.C., Moretta L. **Effect of tumor cells and tumor microenvironment on NK-cell function.** Eur J Immunol. 2014; 44(6): 1582-1592.

Vivier E., Tomasello E., Baratin M., Walzer T., Ugolini S. **Functions of natural killer cells**. Nat Immunol. 2008; 9(5): 503-510.

Vyas M., Schneider A.C., Shatnyeva O., Reiners K.S., Tawadros S., Kloess S., et al. **Mono- and dual-targeting triplebodies activate natural killer cells and have anti-tumor activity in vitro and in vivo against chronic lymphocytic leukemia**. Oncoimmunolog. 2016; 5(9): e1211220.

Waldhauer I., Goehlsdorf D., Gieseke F., Weinschenk T., Wittenbrink M., Ludwig A., et al. **Tumor-associated MICA is shed by ADAM proteases**. Cancer Res. 2008; 68(15): 6368-6376.

Waldman A.D., Fritz J.M., Lenardo M.J. **A guide to cancer immunotherapy: from T cell basic science to clinical practice**. Nat Rev Immunol. 2020; 20: 651-668.

Wu J., Gao F.X., Wang C., Qin M., Han F., Xu T., et al. **IL-6 and IL-8 secreted by tumour cells impair the function of NK cells via the STAT3 pathway in oesophageal squamous cell carcinoma**. J Exp Clin Cancer Res. 2018; 38(1): 321.

Wu S.Y., Fu T., Jiang Y.Z., Shao, Z.M. **Natural killer cells in cancer biology and therapy**. Mol Cancer. 2020; 19(1): 120.

Xie G., Dong H., Liang Y., Ham J.D., Rizwan R., Chen J. **CAR-NK cells: A promising cellular immunotherapy for cancer**. EBioMedicine. 2020; 59: 102975.

Yu J., Freud A.G., Caligiuri M.A. **Location and cellular stages of natural killer cell development**. Trends Immunol. 2013; 34(12): 573-582.

Yu J., Wei M., Becknell B., Trotta R., Liu S., Boyd Z., et al. **Pro- and antiinflammatory cytokine signaling: reciprocal antagonism regulates interferon-gamma production by human natural killer cells**. Immunity. 2006; 24(5): 575-590.

Zhang L., Meng Y., Feng X., Han Z. **CAR-NK cells for cancer immunotherapy: from bench to bedside**. Biomark Res. 2022; 10: 12.

Review and Research on Cancer Treatment
Volume 9, Issue 1 (2023)

Companion diagnostics (CDx) – potential to reveal a specific, efficacious therapy for a breast cancer

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ABSTRACT

Precision medicine is a practice related to the individualization of diagnosis and treatment so that the right dose of the right drug can be used at the right time in a given patient. This allows you to shorten diagnosis time, avoid side effects, increase treatment efficacy, improve clinical outcomes, and reduce healthcare costs. Companion diagnostics (CDx) concerns research aimed at identifying and testing optimal biomarkers useful in diagnostics and qualifying a patient for a specific treatment method. Identification of biomarkers is also important in the context of developing new targets and/or new therapies. CDx was tested for oncology applications. Among these, the most targeted drug development efforts include breast cancer (BC), which is the most commonly diagnosed cancer in women (approximately 2.1 million new cases annually; 1/4 of all cancers in women). Over the past 10 years, the incidence of breast cancer in women has increased by more than 20%. Comprehensive breast cancer control covers prevention, early detection, diagnosis, treatment, rehabilitation, and palliative care. Much progress has been made in the diagnosis and treatment of BC. However, it remains a multi-faceted disease that exhibits heterogeneity within the same tumor or different neoplasms, and a variable course of the disease. Expanding knowledge through genetic, proteomic, and metabolomic research on the molecular processes that make up the etiology of cancer allowed for the identification of specific tumor features and the development of targeted therapies against tumors with specific molecular features. Efficient diagnostics or the possibility of predicting the patient's response to treatment is an important goal of modern PM. The concept of co-development of drugs and diagnostics or companion diagnostics (CDx) has emerged, which is now the new horizon of cancer care.

INTRODUCTION

Personalized medicine (PM) is a concept concerning the use of knowledge on the molecular and genetic determinants of diseases to optimize diagnostics and treatment consisting in administering the right dose of a precisely selected drug depending on the individual needs of the patient, at the right time. In the practice of personalized medicine, special emphasis is placed on such attributes of therapy as the type of patient, drug, or dosage. Taking this into account, it can be said that conventional therapy is imprecise – it is based on generalized data on complex populations of patients with a given disease entity, treated in a specific way. Therapy determined in this way often shows lower-than-expected effectiveness. It has long been known that different patients react differently to the same drug and its components. For example, 33% of patients suffering from depression do not respond to antidepressants, and as many as 75% of cancer patients do not respond positively to the same prescribed pharmaceutical. Such patients may not only not benefit from the use but are also at risk of adverse effects. The US Adverse Event Reporting System (FDA Adverse Events Reporting System (FAERS) recorded 5.4 million reports and over a million deaths due to adverse drug reactions second reaction (ADR) in the last 10 years.

Awareness of genetic predisposition to the occurrence of a particular disease contributes significantly to taking actions aimed at diagnosis or implementation of therapy. For example, over 86% of patients who are aware of the genetic predisposition to familial hypercholesterolemia adhere to the prescribed therapy for up to 2 years while having a positive attitude toward the treatment process. Personalization of therapy – in a way that takes into account the patient's genetic characteristics – allows to reduce healthcare costs, and the number of trials and errors in prognosis, while improving clinical outcomes (Frost, 2020).

Personalized medicine is increasingly used in many fields of medicine, including psychiatry, neurology, and cardiology, as well as in oncology – including, among others, in the diagnosis, prevention, and treatment of breast cancer (WHO, 2021).

SEARCH STRATEGY AND SELECTION CRITERIA

A systematic review of the literature was conducted following the Preferred Reporting Items for Systematic reviews like Pubmed. Only 14 articles have been used.

RESULTS OF REVIEW

BREAST CANCER: DEFINITION, EPIDEMIOLOGY, TREATMENT

Breast cancer (BC) is the most common cancer in women. It is formed in the cells lining the ducts (85%) or lobules (15%) of the glandular tissue of the breast. Initially, the cancerous growth is confined to the duct or lobule (*in situ*) without causing symptoms. At this stage, it has minimal potential to spread. However, cancers *in situ* (stage 0) can progress and infiltrate the surrounding breast tissue (invasive breast cancer) and then spread to nearby lymph nodes, forming regional metastases or to other organs in the body, resulting in distant metastases. Extensive metastasis is the most common cause of death (WHO, 2021).

Treatment of breast cancer often consists of a combination of several therapeutic strategies: surgical removal, radiotherapy, and pharmacotherapy (hormone therapy, chemotherapy, and/or targeted biological therapy) used for the spread of cancer from the breast tumor through the blood (WHO, 2021).

There are 2,1 million new cases of the disease recorded annually in the world. In 2020 2.3 million women were diagnosed with breast cancer; 685,000 deaths were related to this cancer. According to data collected at the end of 2020, there were 7.8 million women worldwide who had been diagnosed with breast cancer in the last 5 years. Worldwide, cancer is the largest contributor to the loss of disability-adjusted life years (DALYs) among women with cancer. Breast cancer occurs in every country in the world, regardless of age after puberty. The risk of developing this cancer increases with age. Survival improved in the 1980s in countries that implemented early detection programs combined with various treatment regimens to eradicate invasive diseases (WHO, 2021). Over the last decade, the incidence of breast cancer in low- and middle-income countries has increased by more than 20%. A lower proportion of patients diagnosed with advanced disease in low- and middle-income countries, LMICs), is listed in High-Income countries, HIC). HIC has achieved significant reductions in breast cancer mortality, mainly due to heavy investment in research and advances in early detection and treatment. Known risk factors contributing to the increase in the incidence of breast cancer include changes in reproductive patterns (childbirth, breastfeeding), a sedentary lifestyle, and an unhealthy diet. Cancer control includes prevention, early detection, diagnosis, treatment, rehabilitation, and palliative care. The modern standard of oncological care is characterized by a multidisciplinary team approach. Educational activities developed for healthcare professionals, especially in primary care, can improve diagnosis, treatment, and outcomes and increase the number of appropriate referrals. The involvement of patient support organizations in ensuring adherence to post-operative care from the moment of diagnosis can promote the continuity of treatment. Multidisciplinary cancer care is effective. Oncology councils have the potential to help overcome diagnostic and management barriers in resource-constrained environments where specialists may be less available. An alternative is to partner with regional and private academic centers to run remote cancer councils, allowing for increased access to multidisciplinary expertise. Molecular oncology is increasingly becoming part of the standard of care, and molecular cancer teams are expected to become as important as site-specific cancer teams today (Alvarado, 2021). When a multidisciplinary approach is unavailable, patients first consult their GP. Only then does a surgical consultation follow. The presence of the tumor is confirmed histopathologically, which precedes the establishment of a treatment plan by a clinical oncologist or surgeon. Early detection of cancer improves patient outcomes because it affects the range of treatment options and contributes to extending the life and improving its quality (WHO, 2021). In addition, early identification of predispositions and genetic conditions may help in taking preventive measures and influence the early start of treatment in the event of a diagnosis. It is worth mentioning that a patient in the advanced stage of the disease generates much higher healthcare costs than a patient in the first stage. Thus, the earlier the diagnosis is made, the more likely it is that treatment will be started at an early stage, where the cancer is more amenable to treatment. Early detection of the disease also helps to prevent early mortality. High-quality mammography examinations, their proper targeting, and their frequency allow for achieving optimal benefits in screening programs (Alvarado, 2021). An integrated approach to the treatment of breast cancer patients can help mitigate the adverse effects of treatment and improve survival. This is most easily achieved by disseminating education to clinicians, who then pass on recommendations to their patients (Pelosci, 2022). Treatment of breast cancer can be very

successful, with a survival rate of 90% or more, especially if the disease is diagnosed early. While all breast cancers used to be treated surgically by mastectomy (total removal of the breast), a mastectomy may now be required if a large tumor is found. A lumpectomy or partial mastectomy is a procedure in which only the tumor and surrounding healthy tissue are removed from the breast. In these cases, radiotherapy to the breast is generally required to minimize the risk of recurrence. In the case of invasive cancer, lymph nodes are removed during cancer surgery. Procedures on smaller lymph nodes, i.e. 'sentinel node biopsies', with relatively few complications, are preferred. Breast cancer chemotherapy does not require hospitalization in the absence of complications. In the early stages, irradiation (radiotherapy) may prevent the need for a mastectomy. For late-stage cancer, radiation therapy can reduce the risk of cancer coming back, even after a mastectomy. In advanced breast cancer, in certain circumstances, radiotherapy can reduce the chance of dying from the disease. The effectiveness of breast cancer therapy depends on the correct course of treatment (WHO, 2021).

PERSONALIZED MEDICINE IN BREAST CANCER

In recent years, tremendous progress has been made in the treatment of breast cancer. Personalized medicine plays an increasingly important role in cancer prevention, diagnosis, treatment, and prognosis (Jackson, 2015). This cancer is a multifaceted disease that exhibits heterogeneity and fluctuating course. Knowledge about the molecular processes that contribute to the etiology of cancer is increasing. This allows the identification of specific features of the tumor and the development of targeted therapies targeted at specific cancers with specific molecular characteristics. Therefore, the goal of personalized medicine is the ability to predict the individual response to specific therapy and adjust the method of treatment to the individual characteristics of the patient and the characteristics of cancer cells. The proven effectiveness of targeted therapies in various tumors suggests that personalized medicine should be promoted as giving the best results.

ACCOMPANYING DIAGNOSIS – DEFINITION, BENEFITS DIAGNOSIS, AND TREATMENT

In oncology, the concepts of drug development in conjunction with companion diagnostics based on cancer-derived DNA tests are important (companion diagnostic, CDx), isolated from peripheral blood. Such concepts fit into pharmacogenomics. This, in turn, is based on the use of a person's genomic structure to predict the response to a drug or to tailor therapy specifically for a given patient. CDx is described as a medical device (often an *in vitro device*) that provides the information necessary for the safe and effective use of an appropriate drug (Alvarado, 2021) or biological product (U.S. Food & Drug Administration, 2021; Frost, 2020). CDx is understood and applied according to specific genomic, and molecular findings identifying patients likely to respond to targeted therapy and stratifying patients according to the molecular profile of a particular disease (Alvarado, 2021). Several CDx tests exist for oncology applications. In 2017, at least 387 targeted drugs were developed or marketed in oncology in the United States. Among these, the most targeted actions were those for breast cancer, non-small cell lung cancer, and colorectal cancer. CDx also improves the selection of participants for clinical trials (Frost, 2020).

The difficulty in obtaining tissue samples is a current challenge for accurate and timely oncology diagnostics. Therefore, liquid biopsy is becoming an increasingly used diagnostic tool. The undoubted advantage of this solution is minimal invasiveness and the fact that this method directly detects circulating tumor cells, cell-free circulating tumor DNA (ctDNA), or extracellular vesicles. This undoubtedly promotes accurate diagnosis (Frost, 2020). The use of accompanying diagnostics allows you to control the growing health expenses. This is due to the possibility of limiting the use of drugs only to those whose effects will be beneficial while reducing the costs associated with side effects. CDx has a positive effect on patient safety by reducing the frequency of invasive procedures. CDx can also improve the predictability of the oncology drug development process, as it enables better selection of the target population and lower research costs. The added value throughout the process is the ability to collect useful data (Alvarado, 2021).

MOLECULAR TESTS IN ACCOMPANYING DIAGNOSTICS

Hybridization *in situ* (*In situ hybridization*, ISH), quantitative real-time polymerase chain reaction (qRT PCR), and immunohistochemistry (IHC) are used to diagnose or detect the relevant disease biomarker. Next-generation sequencing, real-time single-molecule DNA sequencing, digital pathology, and quantitative histopathology have particularly influenced the development of CDx. Quantitative histopathology and digital pathology are medical imaging-based diagnostic approaches. For example, they measure protein biomarkers

in a tissue sample, which are identified and quantified using an automated fluorescence-based imaging platform (Frost, 2020). The combination of a study aimed at assessing the HER2 predictor factor (human epidermal growth factor receptor 2) in patients diagnosed with breast cancer, CDx, and trastuzumab treatment is an example of the successful implementation of CDx in Los Angeles (LA). This combination is cost-effective for healthcare systems and improves patient survival. The therapeutic use of molecular biomarkers is based on their detection and quantification. However, there are problems associated with this. For example, IHC testing is the standard method for detecting tumor biomarkers due to its ease of use, accessibility, use of routine microscopy, and ability to archive stained slides. However, in LA, IHC validation processes are not standardized and quality control is not common practice in laboratories. *In situ* nucleic acid hybridization (ISH) techniques are only available in highly specialized laboratories and institutes. They consist in attaching a labeled polynucleotide to complementary DNA sequences in the cell. The development of biosimilar tests requires a long validation process, which increases the cost of the tests and extends the delay in the availability of tests (Alvarado, 2021).

DIAGNOSTICS ACCOMPANYING BREAST CANCER

In breast cancer, therapeutic decisions must take into account both the biological subtypes of the tumors and their molecular and genetic characteristics; it is important to consider the risk of recurrence in patients with early breast cancer. Current methods for determining the risk of recurrence in this patient population are based on staging and are carried out according to standard clinical and pathological features of the disease. In particular, these features may not reflect the full risk of recurrence in people with early breast cancer (Hurvitz, 2022).

RECEPTORS: ESTROGEN AND PROGESTERONE

Cancers that express the estrogen receptor (ER) and/or the progesterone receptor (PR) are likely to respond to endocrine therapies (hormones) involving substances such as tamoxifen or aromatase inhibitors. These drugs are taken by mouth for 5-10 years and reduce the chance of recurrence of these ' hormone-positive ' tumors by almost half. Endocrine therapies may cause menopausal symptoms but are generally well tolerated. Cancers that do not express ER or PR are "hormone receptor negative" and require chemotherapy treatment unless the cancer is very small (WHO, 2021).

HER-2

Breast cancers can independently overexpress a molecule called the HER-2/ neu oncogene. "HER-2 positive" tumors are amenable to therapy with targeted biological drugs, e.g. trastuzumab. These biological agents are very effective but also expensive. Targeted biological therapies are often combined with chemotherapy to increase the effectiveness of killing cancer cells (WHO, 2021).

The success stories of trastuzumab and endocrine therapy in patients with HER2-positive and HR-positive BC show the potential of PM in the treatment of cancer. The current list of FDA-approved CDx remains almost exclusively based on molecular targets. In BC, most are based on protein detection with IHC. The over-expression of HER2 and the presence of the HER2 protein on the cell surface make HER2 an ideal molecule for use in targeted therapy. Trastuzumab was the first FDA-approved biologic for the treatment of HER2-positive BC. The use of the drug improves survival in 50% of neoadjuvant, adjuvant, and metastatic cases (Alvarado, 2021). HER2 testing is recommended for all primary, metastatic, and recurrent BC due to its high prognostic and predictive value. There are currently seven U.S. Food and Drug Administration (FDA) approved CDx kits to detect HER2 in BC *in vitro*.

For the FD A-approved HER2 breast cancer diagnostic test that also uses IHC CDx. To reduce interlaboratory variability, the American Society of Clinical Oncology (ASCO) in collaboration with the College of American Pathologists (CAP) has published recommendations and guidelines for HER2 IHC staging and scoring (Alvarado, 2021).

HERCEPTEST IN RESEARCH CLINICAL CONCERNING CANCER BREASTS (HERA, CLEOPATRA, EMILIA, ETC.)

The development of a drug that inhibits the proliferation of cancerous cells was one of the most important projects implemented in the 20th century. This anti-cancer drug is trastuzumab. The challenge for the development of trastuzumab concerned the selection of the right group of patients who are likely to respond

to the drug. In this case, the availability of a robust, accurate, and reliable test to detect HER2 overexpression in tumor cells was crucial.

In the clinical development of trastuzumab, the CTA (Clinical Trial Assistant), which was developed by Genentech, was used to select HER2-positive patients. It was only in the phase III trial with trastuzumab that the new IHC test, HercepTest ™, was optimized. HercepTest ™ was designed and developed by Dako. During the final development phase of HercepTest ™, a comparative study was conducted between HercepTest ™ and the CTA test. The goal was to indicate agreement between the two tests. In 1998, HercepTest ™ and the trastuzumab Test were simultaneously approved. HercepTest ™ has been used to screen cancer patients in several major breast cancer clinical trials. In these trials, HercepTest ™ has been used in the clinical stages of adjuvant, neoadjuvant, and metastatic therapy, and for many different types of HER2-targeted therapies. The HercepTest ™ test was also used, among others, in the selection of HER2-positive gastric cancer patients in the ToGA pathway. HercepTest ™ is considered the first companion diagnostic to be approved by the FDA. Over 20 years of using HercepTest ™ have documented the clinical relevance of this diagnostic test (Trost, 2021).

FUTURE DIAGNOSTICS ACCOMPANYING BREAST CANCER

In the oncology literature, theses were indicated that factors such as the patient's age, expression of ER, PR, and HER2 receptors, histological type of cancer and its diameter, degree of histological malignancy or the condition of the regionally located lymph nodes of the patient do not provide sufficient information necessary to plan individually tailored post-operative therapy in the case of breast cancer.

In 2006, an article was published presenting three modern diagnostic tests: CellSearchTM, OncotypeDxTM, and GeneSearchTM. The CellSearch test detects cancer cells circulating in the body of a patient with stage IV breast cancer. The analysis performed with this test helps to determine the prognosis of patients' progression-free survival and overall survival and is more effective than previous standards of care, which included diagnostic imaging studies. The sensitivity of the CellSearch test enables the detection of single cancer cells – it can detect even one cell in a volume of 7.5 ml of a patient's blood. The specificity of the CellSearch test is 99.99%. In studies on a group of patients with cancer progression confirmed by radiological imaging, survival was three times longer among patients with a circulating cancer cell level below 5 than among patients with a circulating cancer cell level above 5 – cells were detected using the CellSearch test. The CellSearch test includes an immunomagnetic technique and identifies circulating tumor-derived tumor cells as EpCAM +, CD45–, and cytokeratin 8,18 and/or 19+.

The OncotypeDX test provides additional data in the form of a score indicating the risk of cancer recurrence. The test allows us to identify the risk of recurrence 10 years ahead (in the case of patients with early-detected breast cancer, at the stage without metastases in regional lymph nodes and positive ER receptors). The impetus for the development of the OncotypeDX test was the analysis of 250 genes, followed by the selection of 21 genes based on the results of reverse transcription polymerase chain reaction (RT-PCR) studies (samples for testing from patients participating in research conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP) (Kołacińska, 2007).

The OncotypeDx test is therefore a 21-gen test that evaluates in a way quantitative the likelihood of breast cancer recurrence. In this way, it supports planning the optimal treatment of patients. In addition, it was noted that among patients included in the NSABP B-20 study, OncotypeDx allowed to correctly predict the effectiveness of adjuvant chemotherapy) (Kołacińska, 2007).

The third of the modern tests – GeneSearch – is designed to detect clinically significant (more than 0.2 mm in size) breast cancer metastases in the material from the removed lymph nodes as soon as possible using two markers: mammaglobin tested using the RT-PCR method and cytocreatine19. The results of the GeneSearch test allow you to make the right decision regarding the performance of total axillary lymphadenectomy, as well as to assess the advancement of the neoplastic disease. The GeneSearch test has become an alternative to the currently used intraoperative methods for examining lymph nodes. A study of 416 patients showed a higher sensitivity of GeneSearch compared to histopathological examinations of imprint biopsy and frozen sections. The GeneSearch test enables very good identification of metastases in cases of lobular breast cancer) (Kołacińska, 2007).

BRCA1 AND BRCA2

Epidemiological and molecular studies have shown a relationship between the risk of breast and ovarian cancer and *BRCA1* and *BRCA2 gene mutations*. This association has been demonstrated and confirmed in a variety of patient groups of different ethnic backgrounds.

The BRCA1 gene encodes a protein that plays an essential role in repairing damaged DNA and regulating the cell cycle during cell division. As a result of mutations in this gene, excessive, uncontrolled cell division and the development of cancer can occur. Mutations in the *BRCA1 gene* increase the likelihood of developing breast, ovarian, prostate, and colorectal cancer. They are inherited in an autosomal dominant fashion and can be passed down from generation to generation.

Women with mutations in the *BRCA1 gene* have an over 80% risk of breast cancer and a 40% risk of ovarian cancer. Cancers associated with the *BRCA1 mutation* account for approximately 10-15% of all breast cancer cases. *BRCA1* mutation carriers also have an increased risk of the fallopian tube and peritoneal cancer, which is about 10%.

BRCA2 encodes a protein that plays an essential role in repairing damaged DNA and regulating the cell cycle. Mutations in this gene can also lead to uncontrolled cell division and tumor development. Mutations are associated with an increased risk of breast, ovarian and gastrointestinal cancers – stomach, colon, and pancreas, in both women and men. It is estimated that mutations in the *BRCA2 gene* increase the risk of breast cancer to 56% and 27% of ovarian cancer.

Identification of the relationship between *BRCA1* and *BRCA2 mutations and* the risk of breast cancer is of great importance in the diagnosis, prevention of cancer, and epidemiological studies (Wang, 2012).

TP53

The TP53 gene is located on chromosome 17. It consists of 10 introns and 11 exons. The product resulting from the translation of the *TP53 gene* is a phosphoprotein, which functions as the main tumor suppressor in the cells of the human body. Phosphoprotein is a transcription factor composed of domains characteristic of known transcription activators. Among these domains, the following can be distinguished: the N-terminal, the C-terminal domain, and the domain responsible for binding to the DNA strand, which performs their respective functions. Currently, at least 12 p53 protein isoforms are known numerous studies are conducted (Guimaraes, 2002). *TP53 gene* mutates in most types of human cancer. It is one of the most frequently analyzed genes in oncology research. The tp53 protein is responsible for tumor growth suppression by regulating cellular repair mechanisms. Loss of tp53 suppressive capacity is caused by autosomal dominant inheritance of *TP53 mutations.* People with *TP53 mutations* show increased susceptibility to cancer, mainly: soft tissue sarcoma, osteosarcoma, breast cancer, adrenal cortex cancer, leukemia, or brain tumors. The p53 protein plays a particularly important role in cells exposed to carcinogens and oncogenic changes (Guimaraes, 2002., Zając, 2015). Thus, the rapid clonal expansion of cells with mutations of the *TP53 gene* may be the initiation of the process of tumor development.

Mutations in the *TP53 gene* are found in nearly 50% of solid tumors. It has been noted that hematological malignancies are less likely to show the presence of mutations within this gene. In the population of patients suffering from hematological malignancies, *TP53* mutations and 17p mutations covering the entire p arm of chromosome 17 are associated with resistance to standard chemotherapy and, consequently, with poor prognosis. Over the last few years, the therapies offered to patients with the *TP53 mutation* are considered to be one of the main challenges facing modern hematology. Mutations in the *TP53 gene* are of great importance in the prognosis and selection of appropriate treatment in patients with chronic lymphocytic leukemia (CCL) (Zając, 2015). Clinical trials of various drugs are underway, which have a chance to directly influence and regulate the activity of the p53 protein or otherwise force the activation of cell cycle regulatory mechanisms. The discovery of such drugs could improve the currently poor prognosis of patients with the *TP53 gene aberration*.

GENES INVOLVED IN CELL CYCLE INHIBITION

The cell cycle consists of the interphase (G_1, S, and G_2 phases), the mitotic phase (mitosis and cytokinesis), and the G_0 phase; which is related to DNA synthesis. Products of various genes from at least three families, such as Cip/Kip, Ink4, and pRb protein families, function as inhibitors of DNA synthesis. They inhibit the

entry of the cell into the S phase of the cell cycle. Ink4 proteins achieve the cell cycle inhibition effect by antagonizing the activation and formation of cyclin (D-CDK4) complexes. Cip/Kip inhibitors achieve their inhibition effect by inhibiting kinases (CDK2). These kinases, in turn, participate in pRb inactivation and, in addition, similarly to cyclin E, probably play other pRb -independent roles. The coordination of the actions of the three classes of proteins mentioned here remains to be explored (Van 't Veer, 2002).

GENE ECPRESSION PROFILING

An important goal of research using molecular techniques, the results of which may have clinical significance, is to link the expression of specific genes with a specific, known cell phenotype. Studies using multiplex molecular probes coupled with fluorescent markers, combined with computer analysis of the results, are of great importance, which allows for the simultaneous visualization of the expression of many genes with high resolution, both in time and space, in single cells of the human body.

MOLECULES INBOLVED IN THE INHIBITION OF IMMUNE CHECKPOINTS

The discovery of immunological checkpoints, such as CTLA-4 or PD-1, had a key impact on the development of new methods of cancer immunotherapy. Initially, these molecules were shown to play an important role in the mechanism of apoptosis and T-cell activation.

The CTLA-4 molecule mainly affects T lymphocytes in the early activation phase within the lymph nodes and acts as a "switch", contributing to the reduction of cytotoxic T lymphocyte activity and limiting autoimmune reactions. Studies conducted on mice deficient in PD-1 or CTLA-4 molecules develop autoimmune-like diseases. These diseases appear quickly after birth and are fatal in cases of CTLA-4 deficiency (if the deficiency is related to PD-1, the symptoms appear much later).

Subsequently, preclinical studies were conducted, the results of which showed the significant role of molecules such as CTLA-4 and PD-1 in maintaining immune tolerance against tumor cells in the periphery.

The development of PD-1 and CTLA-4 blocking methods based on the use of monoclonal antibodies made it possible to restore the normal anti-cancer immune response with the participation of cytotoxic T lymphocytes. So it was amazing that single molecules with PD-1 or CTLA-4 blocking activity showed effective anticancer activity. These discoveries revolutionized cancer immunotherapy. Thanks to the use of such molecules, after many years, for the first time, it was possible to extend the overall survival time of patients with melanoma (Haanen, 2015). Currently, methods of cancer immunotherapy, based on the use of molecules that block immune checkpoints, are used in the treatment of several other cancers, including breast, lung, colon, ovarian, and renal cell carcinoma. Clinical trials are underway, which will probably result in extending the scope of this type of therapy to other types of cancer.

DISCUSSION AND A SHORT CONCLUSION

In recent years, thanks to the implemented projects and clinical trials, it has been possible to design, implement and obtain funding for many effective and innovative methods of cancer therapy. The aim of the actions taken in the field of developing innovative methods of oncological treatment should be to reduce the global mortality rate due to breast cancer by 2.5% per year, thus avoiding 2.5 million deaths due to breast cancer worldwide in the years 2020-2040. Reducing global breast cancer mortality by 2.5% per year would avoid 25% of breast cancer deaths by 2030 and 40% by 2040 among women under 70. The three pillars leading to the achievement of these goals are:

- health promotion for early detection;
- timely diagnosis;
- comprehensive treatment of breast cancer.

Prompt diagnosis can be linked to successful cancer treatment, which in many cases requires specialist oncology care. CDx activities are important, as they provide many therapeutic and prognostic benefits for BC patients. Survival of breast cancer patients for at least 5 years after diagnosis ranges from more than 90% in high-income countries to 66% in India and 40% in South Africa. The developed methods of early detection and treatment of BC have proven effective in high-income countries and should be implemented for use in resource-constrained countries where only some standard diagnostic and therapeutic tools with limited effectiveness are available. In the era of PM, optimal results can be obtained by using the latest methods of diagnosis and treatment, including CDx tests.

References

Alvarado-Cabrero I., Doimi F., Ortega V., Telles de Oliveira Lima J., Torres R., Torregosa L. **Recommendations for streamlining precision medicine in breast cancer care in Latin America**. December 2021; Cancer Reports; issue No. 4; chapter 6.

Frost&Sullivan, **Diagnostics companion in oncology**. Alliance of Advanced BioMedical Engineering; 2020, Available at: https://aabme.asme.org/posts/companion-diagnostics-for-oncology

Guimaraes D., Hainaut P. **TP53: a key gene in human cancer**. Biochimie. 2002; 84(1): 83-93.

Haanen J., Robert C. **Immune Checkpoint Inhibitors**. Immuno-Oncology. 2015; 42: 55-66.

Hurvitz S., Maraka S., Rao R. **Tucatinib for Metastatic HER2+ Breast Cancer and Brain Metastases: Patient Selection**. Oncology. 2022; 4.

Jackson S., Chester J. **Personalized cancer medicine**. Int J Cancer. 2015; 137(2): 262-6.

Kołacińska A., Pawlak M., Morawiec Z. **Novel diagnostic tests for breast cancer**. Oncol, practice. Klin 2007; 3(2): 56-58.

Pelosci A. **Growing Evidence Supports Integrative Care in Breast Cancer**. 2022; Oncology**.**

Trost Jørgensen J., Winther H., Askaa J., Andresen L., Olsen D., Mollerup J. **Companion Diagnostic With Significant Clinical Impact in Treatment of Breast and Gastric Cancer**. 2021.

U.S. Food & Drug Administration. **List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools)**. 2021.

Van 't Veer L., Dai H., Van de Vijver M., He Y., Hart A., Mao M. **Gene expression profiling predicts clinical outcome of breast cancer**. Nature. 2002; 415: 530–536.

Wang F., Fang O., Ge Z., Yu N. **Common BRCA1 and BRCA2 mutations in breast cancer families: a meta-analysis from systematic review**. 39, 2109-2118.

WHO. **Cancer breasts**. 2021; Accessed: 03/26/2021.

Zając M., Giannopoulos K. **TP53 mutations in hematologic malignancies**. Acta Haematol Pol 2015; 46(5): 327-338.

Review and Research on Cancer Treatment
Volume 9, Issue 1 (2023)

Beyond left ventricular ejection fraction. Speckle tracking imaging echocardiography within oncology services

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ABSTRACT

Cardiac oncology is a dynamically changing field in clinical medicine. Oncological therapies are known to cause cardiovascular side effects. Most importantly, it increases the risk of developing heart failure, even years after treatment. The aim of this study was to present novel (last years) data on cardiac imaging methods that are able to show very early cardiac changes due to the toxicity of some chemotherapy drugs.

Rapid developments in the treatment of oncological diseases have led to a constant need for new data. On one hand, new treatment options that affect the heart and vessels are yet to be determined, and more sophisticated treatment planning and modified schemes that make previously used medications or radiotherapeutic options safer for patients are needed. This allows them to be used in patient groups that were previously disqualified because of their burden and risk–benefit ratio. A multidisciplinary team approach, including oncologists and cardiologists with experience in the field, is necessary to manage this complex patient group. Cardiac imaging plays an essential role in the process of risk assessment, diagnosis, and monitoring of cardiac function and morphology during cancer treatment. Echocardiography plays an important role because of its availability and repeatability. Strain imaging is increasingly used in this field. However, multimodal imaging, including magnetic resonance imaging and computed tomography, can provide crucial information to treating specialists. Another topic was the use of implantable cardiac devices in this patient group. Individual decision-making is crucial, as end-of-life care must be taken into clinical consideration. In this paper, we would like to summarise the current state of the art and discuss some novel findings in the field of cardiac oncology which may change the current guidelines

INTRODUCTION

More than 14 million new patients are diagnosed with cancer annually (Parkin, 2005). Due to more effective treatment options the number of surviving patients is steadily increasing. Oncologic therapies are known to cause a variety of cardiovascular side effects, increasing the risk of developing heart failure even years after treatment. In this group of patients, the mortality rate was up to 60% by 2 years (Felker, 2000; Thavendirathan, 2014). Rapid developments in the treatment of oncological diseases have led to a constant need for new data. Cardiac oncology is a dynamically changing field in clinical medicine. On one hand There are new treatment options whose effects on the heart and vessels are yet to be determined; however, more sophisticated treatment planning and modified schemes that make previously used medications or radiotherapeutic options safer for patients are needed. This allows them to be used in patient groups that were previously disqualified because of their burden and risk–benefit ratio. Boer et al. proposed a classification of cardio-oncology syndromes (COS), which is summarized in Table 1 (Boer, 2021). Here, we focused on Type II. A multidisciplinary team approach, including oncologists and cardiologists with experience in the field, is necessary to manage this complex patient group. Cardiac imaging plays an essential role in the process of risk assessment, diagnosis, and monitoring of cardiac function and morphology during cancer treatment. Echocardiography plays an important role because of its availability and repeatability. Strain imaging is increasingly used in this field. However, multimodal imaging, including magnetic resonance imaging and computed tomography, can provide crucial information to treating specialists. Another topic was the use of implantable cardiac devices in this patient group. Individual decision-making is crucial, as end-of-life care must be taken into clinical consideration. In this paper, we would like to summarise the current state of the art and discuss some novel findings in the field of cardiac oncology which may change the current guidelines.

Table 1. The cardio-oncology syndrome (COS) types described by Boer et al.

I	Direct	Progressive development of cancer leads to CV disease
II	Indirect	Cancer associated treatments causing CV disease
III	Direct	Progressive scarring and remodelling of heart and kidney causing a pro-oncogenic environment

IV	Indirect	CV disease associated treatment and diagnostics causing a pro-oncogenic environment
V	Secondary	Systemic and genetic conditions causing both cancer and CV disease

SEARCH STRATEGY AND SELECTION CRITERIA

The literature was reviewed using the PubMed database. Particular attention has been paid to English language articles in recent years. This allowed the gathering of a large number of papers concerning the cardiotoxicity of cancer treatment (including chemotherapy and radiotherapy), as well as monitoring using both biomarkers and imaging studies. The search keywords were cardio-oncology, chemotherapy, radio-therapy, speckle tracking echocardiography, cardiotoxicity, and troponin.

REVIEW

CARDIOTOXIC ONCOLOGICAL THERAPIES

The development of different therapeutic methods and agents in oncology has similarly raised the quality and quantity of the side effects of these methods. Here, we present cardiotoxic media used in chemotherapy and radiotherapy.

The main side effects of cardiotoxic drugs on the circulatory system are as follows:

CHEMOTHERAPY

The list of cardiotoxic side effects of each group of chemotherapeutic agents is long; therefore, we present only a few examples. More detailed information can be found in the guidelines of various cardiac and oncological societies (Zamorano, 2016).

Antimetabolites: Fluoropirimidynes

These agents are known to cause vasospasm and angina symptoms with an incidence of 0,1 to 19% (Iliescu, 2016). The effect is dose dependent. High-dose therapy in the form of intravenous infusion causes vasospasm-related cardiac events in 5,4% of cases (de Forni, 1992; Campia, 2019).

Antimicrotubule agents: Alkaloids

The use of this treatment may lead to chest pain, hypertension, myocardial ischemia, and thromboembolic events (Campia, 2019).

Alkyl like agents: Platinum

The cardiotoxic effects were similar to those of the alkaloids. The use of platinum agents may lead to myocardial infarction (Campia, 2019). Treatment with cisplatin can lead to myocardial ischemia in 0,2-12% of cases (Iliescu, 2016). To avoid platin-related toxicity high volumes are administered. This may lead to volume overload in patients with preexisting cardiac dysfunction (Zamorano, 2016).

Alkylating agents: Cyclophosphamide

Cyclophosphamide rarely causes cardiotoxicity. Described cases are concerning high doses (> 140 mg/kg) before bone marrow transplantation (Braverman, 1991; Zamorano, 2016). They may cause untreatable pulmonary hypertension due to veno-occlusive disease (Ranchoux, 2015; Kim, 2018).

Antitumor antibiotics:

Anthracycline

The most widely described and studied chemotherapeutic agents are known to cause cardiotoxicity. The risk of developing congestive heart failure after doxorubicin administration was 5% at a cumulative dose of 400 mg/m^2 and increased up to 48% when a dose of 700 mg/m^2 was administered (Swain, 2003; Zamorano, 2016). The cardiotoxic effects can be acute, early or late. Acute symptoms develop in < 1% of patients, immediately after drug infusion. Side effects include supraventricular arrhythmia, transient LV dysfunction, and ECG changes, which are usually reversible. Early effects occurred within the first year of treatment and late after several years (Zamorano, 2016).

Bleomycin

Pulmonary hypertension, myocardial ischemia, and even infarction are the most prominent cardiotoxic effects of this drug (Campia, 2019). Moreover, heart failure with elevated NTpro BNP, reduced ejection fraction (EF) and pulmonary oedema are observed.

Antibody related targeted therapy

Trastuzumab induces reduction of LV; therefore, HF is usually reversible with interruption of treatment or administration of HF therapy (Suter, 2007; Zamorano, 2016). Petricciuolo et al. suggest that a level of 14 ng/l of high-sensitivity TnT (hs-TnT) was the best cut off value to predict negative CV outcomes at 3 months in people treated with anti-PD-1/PD-L1 (Petricciuolo, 2020; Delombaerde, 2021).

Tyrosine kinase-related primarily VEGF-R directed

The risk of arterial thromboembolic events increases 3-folds (Scappaticci, 2007; Lenneman, 2016). These agents are also known for causing arterial hypertension (Campia 2019, Saunderson 2021). QT prolongation is a major problem in patients with cancer. Among these agents Vandetanib's average QT prolongation is the longest at 36 ms. QTc intervals of >500 ms occur in 4,3-8% of patients. The US Food and Drug Administration and European Medicines Agency recommend a temporary interruption of treatment when QTc > 500 ms (or the QTc prolongation is > 60 ms above baseline) (Zamorano, 2016).

Tyrosine kinase-related primarily ABL directed

In patients treated with these drugs, the risk of cerebrovascular events, myocardial ischemia, and venous thromboembolic disease increases. Patients may develop precapillary pulmonary hypertension, especially when treated with dasatinib. This side effect occurs in up to 12% of cases (Guignabert, 2016, Montani, 2012, Kim, 2018) Another drug, ponatinib, is associated with systemic hypertension (Campia, 2019).

Proteasome inhibitors

Drugs such as bortezomib and carfilzomib have been reported to cause similar cardiovascular side effects (Campia, 2019).

Immune checkpoint inhibitors

This group of agents may cause myocarditis (Valabhaneni, 2021), which is associated with poor outcomes, as the reported fatality rate ranges from 30 to 50% (Varricchi, 2017, Salem, 2018; Ederhy, 2021). Studies using magnetic resonance found LGE (late gadolinium enhancement – pathological fibrosis and elevated T2-weighted STIR signal with a lymphocytic infiltration) localised in the anteroseptal, inferoseptal, inferior and inferolateral segments of the myocardium (Zhang, 2020, Ederhy, 2021).

RADIOTHERAPY

Approximately 50% of patients with cancer receive radiotherapy during treatment (Baskar, 2012). Modern radiotherapeutic approaches cause less cardiovascular side effects and cardiac damage than before due to more sophisticated radiation schemes, which allow treatment using less radiation on a more focused area – sparing the heart and great vessels. The manifestations of radiation-induced cardiac damage include vasculopathies, pericardial and conduction system, and myocardial and valvular diseases (Desai, 2019).

Research has shown a 34-fold increase in the risk of valvular disease in patients receiving radiotherapy (Heidenreich, 2003; Rosmini, 2021). Anthracycline exposure has been shown to increase the risk of heart failure and valvular disorders from mediastinal radiotherapy, which suggests an additive cardiotoxic effect (Aleman, 2007; Lenneman, 2016).

The incidence of major coronary events increases by 7,4% per mean Gray dose directed to the heart. The characteristic of radiation-induced cardiac damage is that the likelihood of CVD stays increased up to three decades after the initial treatment (Darby, 2013; Bloom, 2016), and in experimental models, cholesterol plaques and thrombosis formed within days after exposure (Stewart 2006; Iliescu 2016). The risk of acute coronary syndrome increases by 16% per Gray in breast cancer patients treated with this method (van den Bogaard, 2017; Rosmini, 2021).

DEFINITIONS OF CARDIOTOXICITY

Cardiotoxicity from cancer therapy includes acute and chronic coronary syndromes, arrhythmias, conduction disturbances, and cancer-therapy-related cardiomyopathy. The latter is based on the left ventricular ejection fraction, which is key information from echocardiography to continue or cease therapy.

Table 2. Different definitions of cardiotoxicity

Source	Definition of cardiotoxicity
FDA (for anthracyclines)	>20% decrease if EF remained normal, or >10% decrease if EF is less than normal
British Society of Echocardiography	LVEF decline by > 10 percentage points to a value of < 50%
Cardiac Review and Evaluation Committee supervising trastuzumab clinical trials	'(1) decrease in cardiac LV ejection fraction (LVEF) that was either global or more severe in the septum; (2) symptoms of congestive heart failure (CHF); (3) associated signs of CHF, including but not limited to S3 gallop, tachycardia, or both; and (4) decline in LVEF of at least 5% to less than 55% with accompanying signs or symptoms of CHF, or a decline in LVEF of at least 10% to below 55% without accompanying signs or symptoms [Curigliano 2012]

CARDIAC MONITORING OF ONCOLOGIC PATIENTS

Clinical strategies for oncological patients should be based on safety and should lead to therapy completion. Therefore, some monitoring methods have been implemented to detect the early side effects of oncotherapy and promptly initiate secondary pharmacological cardioprotection. For those reasons biomarkers and imaging modalities are used. Detailed diagnostic and follow up schemes for different types of chemotherapeutic agents are summarized in the 2022 European Society of Cardiology guidelines on cardio-oncology (Lyon, 2022).

A. Biomarkers.

1. Troponin

The release of cardiac troponins into the bloodstream may damage the cardiac tissue. They are widely available and have comparatively low costs. Testing of this biomarker is not only crucial in monitoring patients during their cancer treatments (Auner 2003), but also allows stratification of the risk, as research shows that elevated baseline levels of troponins are a risk factor for chemotherapy-related complications (Dobson, 2021). In this group of patients closer monitoring of cardiac function may be of benefit (Alvarez-Cardona, 2020). However, the impact on long-term clinical outcomes of routine assessment remains unknown (Yu, 2016; Narayan, 2020).

2. NT-pro BNP

In contrast to troponin, this biomarker is characteristic of cardiac volume overload. It is most widely associated with heart failure. The guidelines of the American Society of Clinical Oncology recommend the use of biomarkers to detect HF only in patients with clinical signs (Armenian, 2017). However the negative predictive value of this test seems to be more useful in clinical practice (Bloom, 2016), and NT-proBNP may also serve as a predictor of clinical outcome and risk of cardiovascular damage during oncologic treatment. Lower baseline levels of it are a predictor of LVEF recovery (Hamo, 2016). It is worth mentioning that certain types of cancer may produce this marker in their vascular endothelium (Narayan, 2020), which may mislead clinicians.

B. Cardiac multimodality imaging strategies

1. 2D echocardiography

Echocardiography with evaluation of the left ventricular ejection fraction (LVEF) remains the paramount image modality in monitoring cancer patients for cardiac damage as the change of this parameter is part of most definitions of cardiotoxicity. According to Dobson et al. most of the echocardiograms in cardio-oncology are performed to monitor treatments using anthracyclines and/or trastuzumab. Lenarczyk et al. showed that echocardiography is the most common method of screening used in 93% of centres implanting

cardiac implantable electronic devices (CIEDs) (Lenarczyk, 2017). The low cost and high availability makes it a good choice for routine repeated control testing. Monitoring of LVEF for 12 months after anthracycline treatment led to early detection of 98% of cardiotoxicity cases (Cardinale, 2016). In the absence of global longitudinal strain (GLS, more information below) quantification of LV longitudinal function using mitral annular excursion (MAPSE) by M-mode echocardiography and/or peak systolic velocity of the mitral annulus by pulsed-wave TDI (tissue Doppler imaging) is recommended (Plana, 2014). These are widely used methods of ultrasound imaging, available in almost every modern ultrasound machine, normally used in daily clinics.

Besides the assessment of the left ventricle it is also crucial to check the systolic function of the right ventricle, as in many cases cancer therapy may impair it function (Plana, 2014). The new British Society of Echocardiography guidelines suggest using all the routinely used parameters including tricuspid annular plane systolic excursion (TAPSE), fractional area change (FAC) and peak systolic velocity (S') of the free wall of the right ventricle to assess the systolic function of the right ventricle (Dobson, 2021). Repeated assessments of all the above mentioned parameters are necessary to notice any relevant changes during oncologic treatment and the observation afterwards.

Full echocardiographic studies of the heart are recommended as they allow diagnosing a variety of cardiotoxic effects besides LVEF reduction and heart failure. Wall motion abnormalities for instance may be a sign of acute coronary syndrome in combination with other findings. As mentioned before the risk of it rises especially during chemotherapy using fluoropyrymidines (Zamorano, 2016, Saunderson, 2021).

Pericardial effusion has also to be evaluated. Screening for signs of tamponade is crucial as it is a life threatening condition.

Chemotherapy may lead to pancytopenia and as a result to sepsis. Echocardiographic studies of cardio-oncologic patients should take endocarditis into clinical consideration and check for vegetations on valves (Plana, 2014). It is important to remember the limitation of transthoracic echocardiography and that transesophageal echocardiography will be often necessary to rule out endocarditis.

All the mentioned above diseases should be diagnosed and treated according to their specific guidelines.

The echocardiographic assessment of an oncologic patient may be challenging and should be performed by an experienced echocardiographer. The cancer treatment itself may alter the image. For example for patients after mastectomies or with breast implants the American Society of Echocardiography suggests using contrast agents for better visualisation of the endocardial border (Mulvagh, 2008; Bloom, 2016).

2. Speckle tracking echocardiography

Speckle tracking echocardiography (STE) is a novel echocardiographic technique, which allows a more precise assessment of systolic function of the left ventricle. Each region of the myocardium has a specific speckle pattern, which allows it to track its movement during the cardiac cycle. Strain defined as the percentage change is then measured in one of the three main dimensions: longitudinal, circumferential, and radial. All of the currently available societies guidelines concerning the echocardiographic evaluation of oncologic patients suggest the use of strain analysis as it provides important information when it comes to treatment outcome (Dobson, 2021, Kim, 2018, Plana, 2014, Virani, 2016). The most studied and used is the longitudinal strain measurement. Global longitudinal strain (GLS) is defined normal

Moreover research suggests that impairment in global longitudinal strain (GLS) may precede LVEF reduction in patients treated using thymidine kinase (TKis) and therefore allow the clinician team to act sooner and prevent negative cardiovascular outcomes (Biersmith, 2022). The new 2022 european guidelines on cardio-oncology are the first to incorporate GLS measurements as a clinical tool (Lyon, 2022). A change of the global longitudinal strain (GLS) > 15% compared to baseline seems to be abnormal and suggests cardiotoxicity (Plana, 2014, Thavendirathan 2014). The Strain Surveillance of Chemotherapy for improving Cardiovascular Outcomes (SUCCOUR) trial has validated the use of GLS in monitoring cardio-oncologic patients undergoing treatment using anthracyclines. Patients monitored using GLS had less meaningful falls of LVEF to the abnormal range compared with the second group monitored using only LVEF (Saunderson, 2021, Thavendiranathan, 2021). Research showed that using myocardial deformation analysis, early epirubicin cardiotoxicity can be detected at a dose of only 200 mg/m^2, which is considered to be low and therefore safe according to current guidelines (Mele, 2015). The British Society of Echocardio-

graphy suggests an echocardiographic classification during surveillance of patients undergoing anthracycline and trastuzumab treatment. Based on LVEF and GLS measurements their change can be defined as: cardiotoxicity, probable subclinical cardiotoxicity and possible subclinical cardiotoxicity. GLS serves here as an indicator of subclinical cardioxic side effects. The definitions are summed up in table 3 (Dobson, 2021).

A study by Negishi et al., suggests that GLS may be used to assess a cardioprotective response after administering beta blockers (Negishi, 2014, Male 2015). The higher sensitivity of GLS compared to LVEF and resulting from it better clinical decision making makes it an essential tool for monitoring cardio-oncologic patients.

Radial strain has not been yet widely studied, however research suggests that it may change earlier and to a greater extent compared to GLS (Jurcut, 2008; Wildiers, 2008, Male, 2015).

Using layer-specific strain analysis showed a change in rotational parameters across different layers of the myocardium (Thavendirathan, 2014).

Speckle tracking analysis of the right ventricle and left atrium can be also performed, however these are less studied techniques. One of the major problems using this technique is inter-vendor variability so results should be compared only between vendors from the same producer. Moreover speckle tracking analysis requires specialistic software, which may not be available. As mentioned before this echocardiographic technique may be challenging and should be performed by a trained echocardiographer. In figure 1 we present a short guide to perform GLS measurements.

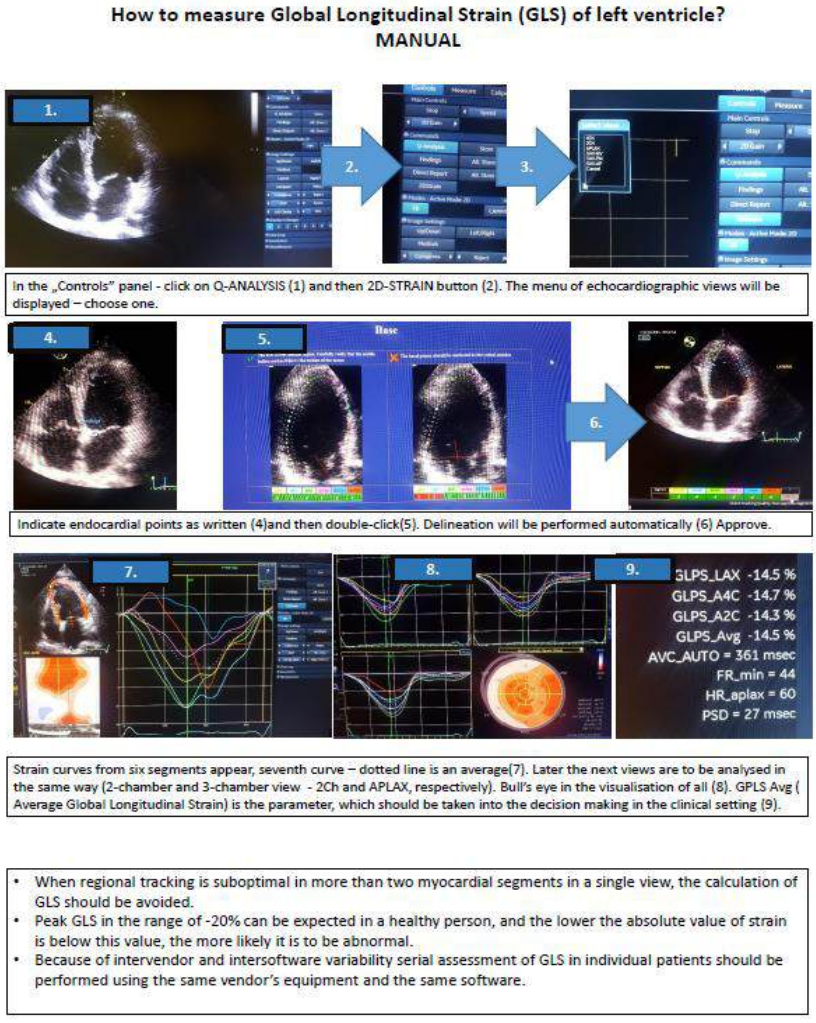

Figure 1. How to measure Global Longitudinal Strain of left ventricle – manual

Table 3. Surveillance criteria proposed by the British Society of Echocardiography (Dobson, 2021)

	Definition
Cardiotoxicity	LVEF decline by > 10 percentage points to a value of < 50%
Probable subclinical cardiotoxicity	LVEF decline by > 10 percentage points to a value of ≥ 50% with an accompanying fall in GLS > 15%
Possible subclinical cardiotoxicity	LVEF decline by < 10 percentage points to a value of < 50% OR GLS relative percentage reduction by > 15% from baseline

3. Cardiac Magnetic Resonance

Magnetic resonance of the heart remains the gold standard to evaluate left and right ventricular systolic function. Typically it is used when echocardiographic assessment is not possible (Zamorano, 2016). The previously mentioned study by Lenarczyk et al. showed that cardiac MRI was used in only 13% of centres implanting cardiac implantable electronic devices (CIED's) for cardio-oncologic screening (Lenarczyk, 2017). The lower availability of this imaging study makes it less useful in everyday clinical practice, however when used it provides very precise information thanks to tissue characterization, which may be crucial for example in detecting myocarditis. The change of cardiomyocyte size which can be assessed using MR as the basis of reduction of LV mass is studied (Saunderson, 2021). Cardiac MR may also be used to evaluate the valves, but echocardiography is more accessible, which allows repeatability.

4. Cardiac computed tomography

Thanks to modern advances in imaging technology the radiation used for computed tomography is lowered – this is important especially in the setting of oncologic patients, which are already exposed to high doses of it during their treatment. The average cardiac CT delivers a lower effective radiation dose of 2–5 vs. 6–21 mSv for single-photon emission CT and 2–20 mSv for invasive angiography (Rosmini, 2021). This allows the use of cardiac computed tomography in the evaluation and diagnosis of cardio-oncologic patients. The main use of cardiac computed tomography in patients undergoing oncologic treatment is screening for coronary artery disease (CAD), by determining the coronary artery calcium score, especially in patients with a higher bleeding risk due to their disease, where traditional coronary angiography would be contraindicated (Biersmith, 2022). A score of 400 agatston units and higher is associated with a higher risk of acute coronary events. Negative values suggest a very low risk of such events (Hecht, 2015, Rosmini, 2021). Current guidelines recommend screening for CAD 5-10 years after radiotherapy (Lancellotti, 2013, Rosmini 2021). It is important to remember that this group of patients may be asymptomatic in terms of angina symptoms due to nervous damage caused by chest irradiation, so special precaution is needed (van Leuwen, 2011; Rosmini, 2021).

Another important application of this technique in this clinical setting is the evaluation of pericardial disease, which, as mentioned before, may be caused by radiotherapy but also certain chemotherapeutic agents.

The role of valvular diagnostics using CCT is limited, as echocardiography will be the method of choice in most cases.

With the rapid development in this field of radiology (for example quantification of epicardial and pericoronary fat) there may be more indications for CCT in cardio-oncology the nearest future (Rosmini 2021).

Table 4. Comparison of the available imaging modalities

	Function assessment	**Tissue characte-rization**	**Availability**	**Cost**	**Myocarditis**	**Pericardial disease**	**Valve disease**	**Coronary disease**
Echocardiography	++	+	+++	+++	+	+	+++	+

Cardiac computed tomography	+	+	++	++	-	++	+	+++
Cardiac magnetic resonance	+++	+++	+	+	+++	+++	++	++

ONCOLOGIC PATIENTS WITH CARDIAC IMPLANTABLE DEVICES

An European Heart Rhythm Association (EHRA) survey from 2017 showed that 89% of centres implanting cardiac implantable electronic devices (CIED's) are managing patients treated for oncologic diseases (Lenarczyk, 2017). According to a Danish survey the annual rate of radiotherapy in patients with CIED was 4,33 therapies per 100 000 persons (Zaremba, 2015; Stuhlinger, 2022). CIED's are almost never a contra-indication for radiotherapy, as it is mostly possible to perform it while keeping the generator outside the beam range. Negative effects described in literature are limited to single case reports. The group at highest risk are patients with pacemaker dependency or ICD's, so these cases require special consideration. If the cumulative dose is more than 5 Gy or the radiation beam energy greater than 10 MV supervision by a trained cardiologist is recommended (Tajstra 2019). A recent European Heart Rhythm Association (EHRA) consensus document provides more detailed information about this topic (Stuhlinger, 2022).

TREATMENT AND PREVENTION OPTIONS

The target of all monitoring strategies is to decide on the moment of cardioprotective treatment. However, at first – the main emphasis– like in any other patient – should be on the cardiovascular disease risk assessment and control. Secondly some specific preventive medical treatment should be implemented (eg. c. Angiotensin-converting-enzyme inhibitors) (Zamorano, 2016).

A. The risk assessment and control of cardiovascular risk factors

Patients can be assigned to different risk categories according to their pre-treatment risk factors (Tab. 5) (Čelutkienė, 2020). Preventive measures including smoking cessation and control of blood pressure, blood glucose and cholesterol levels are recommended before starting cancer treatment to lower the risk of cardiotoxicity (Cardinale, 2016).

Table 5. Patient-related risk factors considering cardiotoxicity risk (Čelutkienė, 2020)

Risk category	Patient-related factor
Low	Age > 18 < 50 years
Medium	Age 50 - 64 years 1 -2 CV risk factors (hypertension, dyslipidemia, obesity, insulin resistance, smoking)
High	Age 65 years > 2 CV risk factors Diabetes Underlying CV disease: CAD, PAD, CMP, severe VHD, heart failure, Reduced or low-normal LVEF (50-54%) pre-treatment Prior cancer therapy

CV – cardiovascular, CAD – coronary artery disease, PAD – peripheral artery disease, CMP – cardiomyopathy, VHD – valvular heart disease

B. Dexrazoxane

Dexrazoxane is a protectant which may be used during anthracycline treatment. The mechanism of action is thought to be iron chelating and thereby decreasing the production of free radicals, which would damage the heart (Jones, 2008; Hamo, 2016). The American Society of Clinical Oncology recommends the use of dexrazoxane only in adult patients with metastatic breast cancer and other malignancies who have received >300 mg/m^2 and who may benefit from use of additional anthracyclines (Hensley 2009; Hamo, 2016).

C. Angiotensin-converting-enzyme inhibitors (ACEI)

Enalapril may be started when myocardial injury is detected through elevated troponin levels (Cardinale, 2016). A combination of ACEI and BB is recommended in the treatment of reduced LVEF without symptoms of heart failure (Cardinale, 2016). The results of the OVERCOME trial showed that a combination of carvedilol and enalapril preserved the LVEF of patients undergoing anthracycline treatment (Bosch, 2013; Cardinale, 2016).

D. Beta-blockers

They may be used in primary prevention (Cardinale, 2016). Like previously stated a combination with ACEI should be prescribed once a reduction in LVEF is detected, even without symptoms of heart failure (Cardinale, 2016). Non-selective beta-blockers like propranolol however may be cardiotoxic (Choe, 1978; Cardinale, 2016). The PRADA trial (Prevention of Cardiac Dysfunction During Adjuvant Breast Cancer Therapy), a randomised, placebo-controlled, double-blind study tested if candesartan or metoprolol can prevent a decline in LVEF due to breast cancer chemotherapy using epirubicin, with or without trastuzumab. It reported that only candesartan showed this effect (Gulati, 2016). The LVEF-change was assessed using cardiac magnetic resonance, which makes this result even more important. These may indicate that not all beta blockers should be used in this indication. Metoprolol seems to be neutral (Cardinale 2016). The previously mentioned OVERCOME study may suggest that carvedilol is effective (Bosch, 2013). The multivariate analysis of the MANTICORE-101 study showed a preservation of LVEF associated with bisoprolol and perindopril (Pituskin 2017). These findings show that beta blockers seem to be more effective in combination with ACEI.

E. Aldosterone antagonists

Akpek et al. found in a study with 83 patients treated because of breast cancer that aldosterone antagonists prevented LVEF reduction (Akpek, 2015). Some studies suggest that this group of drugs can attenuate trastuzumab induced cardiotoxicity through inhibition of the EGFR receptor (Hamo, 2016).

F. Statins

Statins may be beneficial in the clinical setting of cardio-oncology thanks to their pleiotropic effects (Hamo, 2016). In patients without preexisting cardiovascular burden, the use of atorvastatin allowed a higher preservation of LVEF, which was shown in the only, to our knowledge, clinical trial concerning the use of statins for this indication (Acar, 2011; Cardinale, 2016; Hamo, 2016). In animal models pre-treatment using fluvastatin and lovastatin showed positive effects (Riad, 2009; Henninger, 2015; Cardinale, 2016). It should be noted that the use of statins in combination with hepatotoxic chemotherapy or in patients with impaired liver function should be avoided (Iliescu, 2016).

G. Cardiac rehabilitation

Current research indicates that cancer survivors may benefit from exercise in terms of the previously discussed cardiotoxic side effects. In animal models aerobic exercise reduced the cardiotoxic effect of doxorubicin treatment (Cardinale, 2016). However larger studies, including randomised control trials (RCT's) are needed to confirm these findings (Gilchrist, 2019).

Pareek et al suggested a classification of cardiotoxic effects of cancer treatment with appropriate management strategies, in the sense of cancer as well as cardiologic therapy, which we summarised in table 6 (Pareek, 2018).

Table 6. Management strategies for cardiotoxic cancer treatment according to Royal Brompton Hospital myocardial toxicity class

Cardiotoxicity group	Classification	Definition		Management strategies	
		Biomarkers	Echocardiographic findings	Oncology therapy	Cardiology therapy
1	Early biochemical cardiotoxicity	Rise of BNP or troponin I above norm or > 20%	Normal imaging	Continue	Cardiooncology review. Consider closer monitoring or

2	Early functional cardiotoxicity	Normal	New reduction in GLS OR III-IV diastolic dysfunction		start low dose ACEI or BB cardioprotection
3	Early mixed cardiotoxicity	Abnormal	Normal LVEF with GLS or diastolic dysfunction		
4	Symptomatic HFpEF		Symptomatic HFpEF	Interrupt and review risk/benefit	Cardiooncology review. Diuretic for fluid congestion. ACEI or BB cardioprotection if continuing cancer therapy
5	Asymptomatic LVSD		New LVEF reduction to <50% or a reduction of >10% to <55%	Review and balance risk/benefit	Cardiooncology review. Start ACEI and/or BB and up-titrate to 50-100% target dose for HF as tolerated.
6	Symptomatic LVSD		Symptomatic LVEF reduction to <50% or a reduction of >10% to <55%	Interrupt and review risk/benefit	Cardiooncology review. Start ACEI and/or BB and up-titrate to 100% target dose for HF as tolerated.

HFpEF – heart failure with preserved ejection fraction, LVSD – left ventricular systolic dysfunction

DISCUSSION

As mentioned in the introduction the connection between cardiology and oncology is more complex than just treatment side effects. Research suggests that cardiologic conditions may predispose to certain cancer types (Boer, 2021). Heart failure showed prooncogenic effects in animal models (Meijers, 2018). When it comes to cardiotoxic side effects of cancer treatments there are mostly observational retrospective studies. They do not cover every type of agent equally. The prevalence of cancer treatment induced heart failure (HF) is most likely underestimated, as the population of cancer trials are usually younger and healthier than the patients one may encounter in everyday practice (Hamo, 2016).

A multidisciplinary team approach, including oncologists and cardiologists with experience in the field, is necessary to manage this complex patient group. There is no specialty or subspecialty for this medical field for now. Some medical societies are working on determining how training in this field should look like. Leihan et al suggest that centres offering such program should allow the trainee a minimum of 100 patients encounters per year (Lenihan, 2016).

The COVID-19 pandemic had an impact also on the field of cardio-oncology. In a survey by Sadler et al. a decrease in the use of cardiovascular imaging was reported by up to 89% of cardiologists, but only 39% of oncologists. The way in which this particular group of patients, often immunocompromised, is taken care of also changed. More than 85% of the surveyed specialists adopted telemedicine in their everyday clinical practice (Sadler, 2020).

CONCLUSION

Close cooperation, beginning with the diagnostic workup till palliative care decisions, of specialists in the field of oncology as well as cardiology, is essential to ensure optimal patient treatment. Using modern multimodality imaging enables a more detailed and precise diagnostic of cardiotoxicity. Combined with widely used cardiac biomarkers as well as newly studied ones this allows clinical staging and optimal therapy. Pharmaceuticals which were already used in cardiology, show in an increasing number of studies cardioprotective properties in this specific group of patients. This makes it possible to avoid harmful side effects of effective treatment options. Rehabilitation, widely studied in cardiovascular disease management, seems to have positive effects here as well. Cardio-oncology is a novel, but rapidly developing field of medicine. Despite the growing amount of data, there are still knowledge gaps and the lack of randomised

controlled trials and clear guidelines based on their findings make clinical decision making more challenging. A multidisciplinary team approach with experienced physicians specialising in this field of medicine is essential. For now there is no subspecialty or formalised training in cardio-oncology, but experienced centres offer programmes. The first European guidelines in this field were published in 2022 (Lyon, 2022).

References

Acar Z., Kale A., Turgut M., Demircan S., Durna, K., Demir S., et al. **Efficiency of atorvastatin in the protection of anthracycline-induced cardiomyopathy**. Journal of the American College of Cardiology. 2011; 58(9): 988-989. doi:10.1016/J.JACC.2011.05.025.

Akpek M., Ozdogru I., Sahin O., Inanc M., Dogan A., Yazici C., et al. **Protective effects of spironolactone against anthracycline-induced cardiomyopathy**. Eur J Heart Fail. 2015; 17(1): 81-89. doi:10.1002/EJHF.196.

Aleman B.M.P., Van Den Belt-Dusebout A.W., De Bruin M.L., Van 't Veer M.B., Baaijens M.H.A., de Boer J.P., et al. **Late cardiotoxicity after treatment for Hodgkin lymphoma**. Blood. 2007; 109(5): 1878-1886. doi:10.1182/BLOOD-2006-07-034405.

Alvarez-Cardona J.A., Zhang K.W., Mitchell J.D., Zaha V.G., Fisch M.J., Lenihan D.J. **Cardiac Biomarkers During Cancer Therapy: Practical Applications for Cardio-Oncology**. JACC CardioOncol. 2020; 2(5): 791-794. doi:10.1016/J.JACCAO.2020.08.014.

Armenian S.H., Lacchetti C., Barac A., Carver J., Constine L.S., Denduluri N., et al. **Prevention and monitoring of cardiac dysfunction in survivors of adult cancers: American society of clinical oncology clinical practice guideline**. J Clin Oncol. 2017; 35(8): 893-911. doi:10.1200/JCO.2016.70.5400.

Auner H.W., Tinchon C., Linkesch W., Tiran A., Quehenberger F., Link H., et al. **Prolonged monitoring of troponin T for the detection of anthracycline cardiotoxicity in adults with hematological malignancies**. Ann hematol. 2003; 82(4): 218-222. doi:10.1007/S00277-003-0615-3.

Baskar R., Lee K.A., Yeo R. and Yeoh K.W. **Cancer and Radiation Therapy: Current Advances and Future Directions**. Int J Med Sci. 2012; 9(3): 193. doi:10.7150/IJMS.3635.

Biersmith, M.A., Tong, M.S., Guha, A., Simonetti, O.P., Addison D. **Multimodality Cardiac Imaging in the Era of Emerging Cancer Therapies**. J Am Heart Assoc. 2020; 9(2). doi:10.1161/JAHA.119.013755.

Bloom M.W., Hamo C.E., Cardinale D., Ky, B., Nohria A., Baer L., et al. **Cancer Therapy-Related Cardiac Dysfunction and Heart Failure: Part 1: Definitions, Pathophysiology, Risk Factors, and Imaging**. Circ Heart Fail. 2016; 9(1). doi:10.1161/CIRCHEARTFAILURE.115.002661.

Bosch X., Rovira M., Sitges M., Domènech A., Ortiz-Pérez J.T., de Caralt T.M., et al. **Enalapril and carvedilol for preventing chemotherapy-induced left ventricular systolic dysfunction in patients with malignant hemopathies: the OVERCOME trial (preventiOn of left Ventricular dysfunction with Enalapril and caRvedilol in patients submitted to intensive ChemOtherapy for the treatment of Malignant hemopathies)**. J Am Coll Cardiol. 2013; 61(23): 2355-2362. doi:10.1016/J.JACC.2013.02.072.

Braverman A.C., Antin J.H., Plappert M.T., Cook E.F., Lee R.T. **Cyclophosphamide cardiotoxicity in bone marrow transplantation: a prospective evaluation of new dosing regimens**. J Clin Oncol. 1991; 9(7): 1215-1223. doi:10.1200/JCO.1991.9.7.1215.

Campia U., Moslehi J.J., Chair V., Amiri-Kordestani L., Barac A., Beckman J.A., et al. **On behalf of the American Heart Association Council on Peripheral Vascular Disease; Council on Arteriosclerosis, Thrombosis and Vascu-lar Biology; and Council on Cardiovascular and Stroke Nursing Cardio-Oncology: Vascular and Metabolic Perspectives A Scientific Statement From the American Heart Association**. Circulation. 2019; 139: 579-602. doi:10.1161/CIR.0000000000000641.

Cardinale D., Biasillo G., Cipolla C.M. **Curing Cancer, Saving the Heart: A Challenge That Cardioncology Should Not Miss**. Current Cardiology Reports. 2016; 18(6): 1-10. doi:10.1007/S11886-016-0731-Z.

Čelutkienė J., Pudil R., López-Fernández T., Grapsa J., Nihoyannopoulos P., Bergler-Klein J., et al. **Role of cardiovascular imaging in cancer patients receiving cardiotoxic therapies: a position statement on behalf of the Heart Failure Association (HFA), the European Association of Cardiovascular Imaging (EACVI) and the Cardio-Oncology Council of the European Society of Cardiology (ESC)**. Eur J Heart Fail. 2020; 22(9): 1504-1524. doi:10.1002/EJHF.1957.

Choe J.Y., Combs A.B. and Folkers K. **Potentiation of the toxicity of adriamycin by propranolol**. Res Comun Chem Pathol Pharmacol. 1978; 21(3): 577-80. Available at: http://www.ncbi.nlm.nih.gov/pubmed/705032.

Darby S.C., Ewertz M., McGale P., Bennet A.M., Blom-Goldman U., Bronnum D., et al. **Risk of Ischemic Heart Disease in Women after Radiotherapy for Breast Cancer**. N Engl J Med. 2013; 368(11): 987-998. doi:10.1056/NEJMOA1209825/SUPPL_FILE/NEJMOA1209825_DISCLOSURES.PDF.

de Boer R.A., Aboumsallem J.P., Bracun V., Leedy D., Cheng R., Patel S., et al. **A new classification of cardio-oncology syndromes**. Cardio-Oncology. 2021; 7(1). doi:10.1186/S40959-021-00110-1.

Delombaerde D., Vervloet D., Franssen C., Croes L., Gremonprez F., et al. **Clinical implications of isolated troponinemia following immune checkpoint inhibitor therapy**. ESMO Open. 2021; 6(4): 100216. doi:10.1016/J.ESMOOP.2021.100216.

Desai M.Y., Windecker S., Lancellotti P., Bax J.J., Griffin B.P., Cahlon O., et al. **Prevention, Diagnosis, and Management of Radiation-Associated Cardiac Disease: JACC Scientific Expert Panel**. J Am Coll Cardiol. 2019; 74(7): 905-927. doi:10.1016/J.JACC.2019.07.006.

Dobson R., Ghosh A.K., Ky B., Marwick T., Stout M., Harkness A., et al. **British Society for Echocardiography and British Cardio-Oncology Society guideline for transthoracic echocardiographic assessment of adult cancer patients receiving anthracyclines and/or trastuzumab**. Echo Research and Practice. 2021; 8(1): G1–G18. doi:10.1530/ERP-21-0001.

Dobson R., Ghosh A.K., Ky B., Marwick T., Stout M., Harkness A.et al. **BSE and BCOS Guideline for Transthoracic Echocardiographic Assessment of Adult Cancer Patients Receiving Anthracyclines and/or Trastuzumab**. JACC: CardioOncology. 2021; 3(1): 1-16. doi:10.1016/J.JACCAO.2021.01.011/SUPPL_FILE/MMC1.PDF.

Ederhy S., Salem J.E., Dercle L., Hasan A.S., Chauvet-Droit M., Nhan P., et al. **Role of Cardiac Imaging in the Diagnosis of Immune Checkpoints Inhibitors Related Myocarditis**. Front Oncol. 2021; 11: 1200. doi:10.3389/FONC.2021.640985/BIBTEX.

Felker G.M., Thompson R.E., Hare J.M., Hruban R.H., Clemetson D.E., Howard D.L., et al. **Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy**. N Engl J Med. 2000; 342(15); 1077-1084. doi:10.1056/NEJM200004133421502.

De Forni M., Malet-Martino M.C., Jaillas P., Shubinski R.E, S, Bachaud J.M., Lemaire L., et al. **Cardiotoxicity of high-dose continuous infusion fluorouracil: a prospective clinical study**. J Clin Oncol. 1992; 10(11): 1795-1801. doi:10.1200/JCO.1992.10.11.1795.

Gilchrist S.C., Barac A., Ades P.A., Alfano C.M., Franklin B.A., et al. **Cardio-Oncology Rehabilitation to Manage Cardiovascular Outcomes in Cancer Patients and Survivors: A Scientific Statement From the American Heart Association**. Circulation. 2019; 139(21): E997-E1012. doi:10.1161/CIR.0000000000000679.

Guignabert C., Phan C., Seferian A., Huertas A., Tu L., Thuillet R., et al. **Dasatinib induces lung vascular toxicity and predisposes to pulmonary hypertension**. J Clin Invest. 2016; 126(9): 3207-3218. doi:10.1172/JCI86249.

Gulati, G., Heck, S.L., Ree A.H., Hoffmann P., Schulz-Menger J., Fagerland M.W., et al. **Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 × 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol**. Eur Heart J. 2016; 37(21): 1671-1680. doi:10.1093/EURHEARTJ/EHW022.

Hamo C.E., Bloom M.W., Cardinale D., Ky B., Nohria A., Baer L. et al. **Cancer therapy-related cardiac dysfunction and heart failure: Part 2: Prevention, treatment, guidelines, and future directions**. Circ Heart Failure. 2016; 9(2). doi:10.1161/CIRCHEARTFAILURE.115.002843.

Hecht H., Blaha M.J., Berman D.S., Nasir K., Budoff M., Leipsic J., et al. **Clinical indications for coronary artery calcium scoring in asymptomatic patients: Expert consensus statement from the Society of Cardiovascular Computed Tomography**. J Cardiovasc Comput Tomogr. 2017; 11(2): 157-168. doi:10.1016/J.JCCT.2017.02.010.

Heidenreich P.A., Hancock S.L., Lee B.K., Mariscal C.S. and Schnittger I. **Asymptomatic cardiac disease following mediastinal irradiation**. J Am Coll Cardiol. 2003; 42(4): 743-749. doi:10.1016/S0735-1097(03)00759-9.

Henninger C., Huelsenbeck S., Wenzel P., Brand M., Huelsenbeck J., Schad A., et al. **Chronic heart damage following doxorubicin treatment is alleviated by lovastatin**. Pharmacol Res. 2015; 91: 47-56. doi:10.1016/J.PHRS.2014.11.003.

Hensley M.L., Hagerty K.L., Kewalramani T., Green D.M., Meropol N.J., Wasserman T.H., et al. **American Society of Clinical Oncology 2008 clinical practice guideline update: use of chemotherapy and radiation therapy protectants**. J Clin Oncol. 2009; 27(1): 127-145. doi:10.1200/JCO.2008.17.2627.

Iliescu C.A., Grines C.L., Herrmann J., Yang E.H., Cilingiroglu M., Charitakis K., et al. **SCAI Expert consensus statement: Evaluation, management, and special considerations of cardio-oncology patients in the cardiac catheterization laboratory (endorsed by the cardiological society of india, and sociedad Latino Americana de Cardiologıa intervencionista)**. Catheter Cardiovasc Interv. 2016; 87(5): E202-E223. doi:10.1002/CCD.26379.

Jones R.L. **Utility of dexrazoxane for the reduction of anthracycline-induced cardiotoxicity**. Expert review of cardiovascular therapy. 2008; 6(10): 1311-1317. doi:10.1586/14779072.6.10.1311.

Jurcut R., Wildiers H., Ganame J., D'hooge J., De Backer J., Denys H., et al. **Strain rate imaging detects early cardiac effects of pegylated liposomal Doxorubicin as adjuvant therapy in elderly patients with breast cancer**. J Am Soc Echocardiogr. 2008; 21(12): 1283-1289. doi:10.1016/J.ECHO.2008.10.005.

Kim H., Chung W.B., Cho K.I., Kim B.J., Seo J.S., Park S.M., et al. **Diagnosis, Treatment, and Prevention of Cardiovascular Toxicity Related to Anti-Cancer Treatment in Clinical Practice: An Opinion Paper from the Working Group on Cardio-Oncology of the Korean Society of Echocardiography**. J Cardiovasc Ultrasound. 2018; 26(1): 1-25. doi:10.4250/JCU.2018.26.1.1.

Lancellotti P., Nkomo V.T., Badano L.P., Bergler-Klein J., Bogaert J., Davin L., et al. **Expert consensus for multi-modality imaging evaluation of cardiovascular complications of radiotherapy in adults: a report from the European Association of Cardiovascular Imaging and the American Society of Echocardiography**. Eur Heart J Cardiovasc Imaging. 2013; 14(8): 721-740. doi:10.1093/EHJCI/JET123.

Lenarczyk R., Potpara T.S., Haugaa K.H., Deharo J.C., Hernandez-Madrid A., et al. **Approach to cardio-oncologic patients with special focus on patients with cardiac implantable electronic devices planned for radiotherapy: results of the European Heart Rhythm Association survey**. Europace. 2017; 19(9): 1579-1584. doi:10.1093/EUROPACE/EUX195.

Lenihan D.J., Hartlage G., DeCara J., Blaes A., Finet J.E., Lyon A.R., et al. **Cardio-Oncology Training: A Proposal From the International Cardioncology Society and Canadian Cardiac Oncology Network for a New Multidisciplinary Specialty**. J Card Fail. 2016; 22(6): 465-471. doi:10.1016/J.CARDFAIL.2016.03.012.

Lenneman C.G., Sawyer D.B. '**Cardio-Oncology**'. Circulation Research. 2016; 118(6): 1008-1020. doi:10.1161/CIRCRESAHA.115.303633.

Lyon A.R., López-Fernández T., Couch L.S., Asteggiano R., Aznar M. Bergler-Klein J., et al. **2022 ESC Guidelines on cardio-oncology developed in collaboration with the European Hematology Association (EHA), the European Society for Therapeutic Radiology and Oncology (ESTRO) and the International Cardio-Oncology Society (IC-OS)Developed by the task force on cardio-oncology of the European Society of Cardiology (ESC)**. Eur Heart J. 2022; 43(41): 4229-4361. doi:10.1093/EURHEARTJ/EHAC244

Meijers W.C., Maglione M., Bakker S.J.L., Oberhuber R., Kieneker L.M., de Jong S., et al. **Heart failure stimulates tumor growth by circulating factors**. Circulation. 2018; 138(7): 678-691. doi:10.1161/CIRCULATIONAHA.117.030816.

Mele D., Rizzo P., Pollina A. V., Fiorencis A., Ferrari R. **Cancer therapy-induced cardiotoxicity: role of ultrasound deformation imaging as an aid to early diagnosis**. Ultrasound Med Biol. 2015; 41(3): 627-643. doi:10.1016/J.ULTRASMEDBIO.2014.11.015.

Montani D., Bergot E., Günther S., Savale L., Bergeron A., Bourdin A., et al. **Pulmonary arterial hypertension in patients treated by dasatinib**. Circulation. 2012; 125(17): 2128-2137. doi:10.1161/CIRCULATIONAHA.111.079921.

Mulvagh S.L., Rakowski H., Vannan M.A., Abdelmoneim S.S., Becher H., Bierig S.M., et al. **American Society of Echocardiography Consensus Statement on the Clinical Applications of Ultrasonic Contrast Agents in Echocardiography**. J Am Soc Echocardiogr. 2008; 21(11): 1179-1201. doi:10.1016/J.ECHO.2008.09.009.

Narayan V., Thompson E.W., Demissei B., Ho J.E., Januzzi J.L., Ky B. **Mechanistic Biomarkers Informative of Both Cancer and Cardiovascular Disease: JACC State-of-the-Art Review**. J Am Coll Cardiol. 2020; 75(21): 2726-2737. doi:10.1016/J.JACC.2020.03.067.

Negishi K., Negishi T., Haluska B.A., Hare J.L., Plana J.C., Marwick T.H., **Use of speckle strain to assess left ventricular responses to cardiotoxic chemotherapy and cardioprotection**. Eur Heart J Cardiovasc Imaging. 2014; 15(3): 324-331. doi:10.1093/EHJCI/JET159.

Pareek N., Cevallos J., Moliner P., Shah M., Tan L.L., Chambers V., et al. **Activity and outcomes of a cardio-oncology service in the United Kingdom-a five-year experience**. Eur J Heart Fail. 2018; 20(12): 1721-1731. doi:10.1002/EJHF.1292.

Parkin D.M., Bray F., Ferlay J., Pisani P. **Global Cancer Statistics, 2002**. CA: A Cancer Journal for Clinicians. 2005; 55(2): 74-108. doi:10.3322/CANJCLIN.55.2.74.

Petricciuolo S., Delle Donne M.G., Aimo A., Chella A., De Caterina R. **Pre-treatment high-sensitivity troponin T for the short-term prediction of cardiac outcomes in patients on immune checkpoint inhibitors**. Eur J Clin Invest. 2021; 51(4): e13400. doi:10.1111/ECI.13400.

Pituskin E., Mackey J.R., Koshman S., Jassal D., Pitz M., Haykowsky M.J., et al. **Multidisciplinary approach to novel therapies in cardio-oncology research (MANTICORE 101-Breast): A randomized trial for the prevention of trastuzumab-associated cardiotoxicity**. J Clin Oncol. 2017; 35(8): 870-877. doi:10.1200/JCO.2016.68.7830.

Plana J.C., Galderisi M., Barac A., Ewer M.S., Ky B., Scherrer-Crosbie M., et al. **Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging**. J Am Soc Echocardiogr. 2014; 27(9): 911-939. doi:10.1016/J.ECHO.2014.07.012.

Ranchoux B., Günther S., Quarck R., Chaumais M.C., Dorfmüller P., Antigny F., et al. **Chemotherapy-Induced Pulmonary Hypertension: Role of Alkylating Agents**. Am J Pathol. 2015; 185(2): 356-371. doi:10.1016/J.AJPATH.2014.10.021.

Riad A., Bien S., Westermann D., Becher P.M., Loya K., Landmesser U., et al. **Pretreatment with statin attenuates the cardiotoxicity of Doxorubicin in mice**. Cancer Res. 2009; 69(2): 695–699. doi:10.1158/0008-5472.CAN-08-3076.

Rosmini S., Aggarwal A., Chen D.H., Conibear J., Davies C.L., Dey A.K., et al. **Cardiac computed tomography in cardio-oncology: an update on recent clinical applications**. Eur Heart J Cardiovasc Imaging. 2021; 22(4): 397-405. doi:10.1093/EHJCI/JEAA351.

Sadler D., DeCara J.M., Herrmann J., Arnold A., Ghosh A.K., Abdel-Qadir H., et al. **Perspectives on the COVID-19 pandemic impact on cardio-oncology: results from the COVID-19 International Collaborative Network survey**. Cardiooncology. 2020; 6(1): 1-13. doi:10.1186/S40959-020-00085-5/TABLES/3.

Salem J.E., Manouchehri A., Moey M., Lebrun-Vignes B., Bastarache L., Pariente A., et al. **Cardiovascular toxicities associated with immune checkpoint inhibitors: an observational, retrospective, pharmacovigilance study**. Lancet Oncol. 2018; 19(12): 1579-1589. doi:10.1016/S1470-2045(18)30608-9.

Saunderson C.E.D., Plein S., Manisty C.H. **Role of cardiovascular magnetic resonance imaging in cardio-oncology**. Eur Heart J Cardiovasc Imaging. 2021; 22(4): 383-396. doi:10.1093/EHJCI/JEAA345.

Scappaticci F.A., Skillings J.R., Holden S.N., Gerber H.P., Miller K., et al. **Arterial Thromboembolic Events in Patients with Metastatic Carcinoma Treated with Chemotherapy and Bevacizumab**. JNCI: Journal of the National Cancer Institute. 2007; 99(16): 1232-1239. doi:10.1093/JNCI/DJM086.

Stewart F.A., Heeneman S., Te Poele J., Kruse J., Russell N.S., Gijbels M., et al. **Ionizing radiation accelerates the development of atherosclerotic lesions in ApoE-/- mice and predisposes to an inflammatory plaque phenotype prone to hemorrhage**. Am J Pathol. 2006; 168(2): 649-658. doi:10.2353/AJPATH.2006.050409.

Stühlinger M., Burri H., Vernooy K., Garcia R, Lenarczyk R, Sultan A., et al., **EHRA consensus on prevention and management of interference due to medical procedures in patients with cardiac implantable electronic devices**. EP Europace. 2022. euac040, https://doi.org/10.1093/europace/euac040

Suter T.M., Procter M., Van Veldhuisen D.J., Muscholl M., Bergh J., Carlomagno C., et al. **Trastuzumab-associated cardiac adverse effects in the herceptin adjuvant trial**. J Clin Oncol. 2007; 25(25): 3859-3865. doi:10.1200/JCO.2006.09.1611.

Swain S.M., Whaley F.S., Ewer M.S. **Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials**. Cancer. 2003; 97(11): 2869-2879. doi:10.1002/CNCR.11407.

Tajstra M., Blamek S., Niedziela J.T., Gadula-Gacek E., Przybylski A., et al. **Patients with cardiac implantable electronic devices undergoing radiotherapy in Poland. Expert opinion of the Heart Rhythm Section of the Polish Cardiac Society and the Polish Society of Radiation Oncology**. Kardiologia polska. 2019; 77(11): 1106-1112. doi:10.33963/KP.15063.

Thavendiranathan P., Negishi T., Somerset E., Negishi K., Penicka M., Lemieux J., et al. **Strain-Guided Management of Potentially Cardiotoxic Cancer Therapy**. J Am Coll Cardiol. 2021; 77(4): 392-401. doi:10.1016/J.JACC.2020.11.020.

Thavendiranathan P., Poulin F., Lim K.D., Plana J.C., Woo A., Marwick T.H. **Use of myocardial strain imaging by echocardiography for the early detection of cardiotoxicity in patients during and after cancer chemotherapy: a systematic review**. J Am Coll Cardiol. 2014; 63(25 Pt A): 2751-2768. doi:10.1016/J.JACC.2014.01.073.

Vallabhaneni S., Zhang K.W., Alvarez-Cardona J.A., Mitchell J.D., Steen H., Woodard P.K., et al. **Role of cardiovascular magnetic resonance in early detection and treatment of cardiac dysfunction in oncology patients**. Int J Cardiovasc Imaging. 2021; 37(10): 3003-3017. doi:10.1007/S10554-021-02271-7.

Van Den Bogaard V.A.B., Ta B.D.P., Van Der Schaaf A., Bouma A.B., Middag A.M.H., Bantema-Joppe E.J., et al. **Validation and Modification of a Prediction Model for Acute Cardiac Events in Patients With Breast Cancer Treated With Radiotherapy Based on Three-Dimensional Dose Distributions to Cardiac Substructures**. J Clin Oncol. 2017; 35(11): 1171-1178. doi:10.1200/JCO.2016.69.8480.

Varricchi G., Galdiero M.R. and Tocchetti C.G. **Cardiac Toxicity of Immune Checkpoint Inhibitors: Cardio-Oncology Meets Immunology**. Circulation. 2017; 136(21): 1989-1992. doi:10.1161/CIRCULATIONAHA.117.029626.

Virani S.A., Dent S., Brezden-Masley C., Clarke B., Davis M.K., Jassal D.S., et al. **Canadian Cardiovascular Society Guidelines for Evaluation and Management of Cardiovascular Complications of Cancer Therapy**. Can J Cardiol. 2016; 32(7): 831-841. doi:10.1016/J.CJCA.2016.02.078.

Wildiers H., Jurcut R., Ganame J., Herbots L., Neven P., De Backer J., et al. **A pilot study to investigate the feasibility and cardiac effects of pegylated liposomal doxorubicin (PL-DOX) as adjuvant therapy in medically fit elderly breast cancer patients**. Crit Rev Oncol Hematol. 2008; 67(2): 133-138. doi:10.1016/J.CRITREVONC.2008.01.001.

Yu A.F., Ky B. **Roadmap for biomarkers of cancer therapy cardiotoxicity**. Heart (British Cardiac Society). 2016; 102(6): 425-430. doi:10.1136/HEARTJNL-2015-307894.

Zamorano J.L., Lancellotti P., Rodriguez Muñoz D., Aboyans V., Asteggiano R., Galderisi M., et al. **2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC)**. Eur Heart J. 2016; 37(36): 2768-2801. doi:10.1093/EURHEARTJ/EHW211.

Zhang L., Awadalla M., Mahmood S.S., Nohria A., Hassan M.Z.O., Thuny F., et al. **Cardiovascular magnetic resonance in immune checkpoint inhibitor-associated myocarditis**. Eur Heart J. 2020; 41(18): 17331743. doi:10.1093/EURHEARTJ/EHAA051.

Review and Research on Cancer Treatment
Volume 9, Issue 1 (2023)

Therapeutic biomaterials – application in neurology and cardiology

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ABSTRACT

Biomaterials are of interest in most medical fields. It's hard to imagine life without them. And due to the ever-increasing demand, scientists are developing new materials. Diseases of the nervous and cardiovascular systems are still a big problem, which are associated with a limited ability to regenerate brain or heart tissues. Therefore, this review discusses the advancement in biomaterial engineering for the treatment of neurological and cardiovascular diseases. Neurodegenerative diseases affect a large percentage of older people. Therefore, the review presents treatment options for Alzheimer's (AD), and Parkinson's diseases (PD). Another serious problem is cardiac ischemia. To regenerate heart tissue, scientists have proposed the use of extracellular vesicles, injectable hydrogels, and biomaterial-based cardiac patches. In addition to tissue engineering, implants are also developing in the field of cardiology. More and more modern materials are being created, e.g., for valve prostheses or vascular stents.

INTRODUCTION

The demand for biomaterials is constantly growing. Biomaterials are used in virtually every field of medicine. Biomaterials science can help create more effective vaccinations, medication delivery systems, and treatments, that can be used to treat or prevent various diseases.

Regardless of their purpose, they must meet several of the most important features. The requirements for the acceptability of biomaterials include technical functionality relating to implant-specific mechanical properties, sufficiently high stability in terms of physiological circumstances, and good biocompatibility. Maintaining biofunctionality over a long period of time is the aim of using biomaterials for implants (Sternberg, 2009). For instance, biomaterials that are administered to the brain must meet several general requirements. The material should correspond to the mechanical properties of the place where it is implanted. Softer materials tend to be less stable and stiffer materials can cause gliosis. It is also important to control the rate of degradation because rapid degradation can impair functionality, but non-degradable materials are associated with chronic inflammation and neuronal loss. The material should not be cytotoxic, immunogenic, or cause excessive secondary damage to nearby cells by causing oedema. A wide range of biomaterials has been used in the brain, e.g. in the form of hydrogels, particles, and electrospun fibers. Hydrogels and particles can be injected into the brain parenchyma, resulting in less trauma, than other forms of surgical implantation, but less structural control, than preformed biomaterials (Lally, 2022). For comparison natural biomaterial has many advantages, i.e. biocompatibility, biodegradability – natural degradation mostly can occur in the body, low toxicity, lower costs, versatility, and integration with cells. But it also provides some disadvantages like low mechanical strength, possible immune reaction, and a risk of contamination. Protein biomaterials (collagen, gelatin, fibrin, albumin, silk fibroin) and polysaccharide biomaterials (hyaluronic acid, alginate, chitosan, heparin, cellulose) are used to repair the brain (Ucar, 2021).

Because cardiomyocytes have a limited capacity to proliferate, cardiovascular disease is a common cause of mortality and morbidity. Cell-based therapies and bioactive molecules are currently used in heart regeneration treatments (Vasu, 2021). Due to the rapid development of materials science and technology, biomaterials have been found to provide biophysical and biochemical cues to regulate the intrinsic regeneration of cardiomyocytes and an external microenvironment conducive to heart repair has recently been identified (Fan, 2023). Cell-based therapies are based on the transfer of a cell suspension to damaged muscle tissue for repairing or replacing it. The supplied cells must be able to differentiate into mature and functional cardiac tissue – it is essential for true cardiac regeneration. Contrary to the development of tissue from directly injected cells, endogenous heart regeneration is made possible by bioactive substances, that harness paracrine actions. For this purpose, growth factors can be used, because they are signaling molecules involved in several biological processes like aging and survival. The delivery of growth factors must be local and scheduled. Endogenous heart regeneration is also possible by using bioactive substances, that harness paracrine actions. Three main methods are leading in cardiac regeneration therapies: extracellular vesicles, injectable hydrogels, and biomaterial-based cardiac patches. When designing material for heart regeneration, particular attention

should be paid to biocompatibility and immunogenicity, and it is important to consider angiogenesis and vascularity to support the contained cells (Vasu, 2021).

This review aims to provide a summary of recent research and challenges on biomaterials for use in neurology and cardiology.

THERAPEUTIC BIOMATERIALS IN NEUROLOGY

Multiple types of disorders, such as neurodegenerative disorders (ND), stroke, and traumatic injury, may damage the central nervous system. Therefore, it is a very difficult problem for doctors to repair their damaged parts. Neurodegenerative disorders refer to progressive damage to neurons leading to functional loss of the nervous system and include, for example, Alzheimer's disease, Huntington's disease (HD), and Parkinson's disease (Akhtar, 2023). For this purpose, scientists have proposed several materials for medicinal purposes.

ALZHEIMER'S DISEASE (AD)

Alzheimer's disease is a multifactorial, irreversible, progressive neurodegenerative disorder. The slow degeneration of brain synapses and nerve cells is an indicator of this disease. The brain's cognitive, memory, and behavioral abilities significantly deteriorate as a result of such persistent and irreversible nerve cell injury (Agrawal, 2021; Hampel, 202; Azargoonjahromi, 2023). AD is a disorder, that becomes worse with age. It contributes to around 60–70% (Agrawal, 2021) of dementia cases worldwide, making it the most prevalent kind of cause of dementia (Agrawal, 2021). Noticeably, the two primary neuropathological markers of Alzheimer's disease are hyperphosphorylated tau and -amyloid, two proteins that accumulate and solidify to form intracellular and extracellular neurofibrillary tangles and plaques, respectively (Azargoonjahromi, 2023). The behavioral symptoms associated with AD include memory loss, cognitive decline, difficulties with learning and thinking, mood changes, problems doing routine activities, etc. A prospective diagnostic and therapeutic tool is urgently needed due to the rising number of cases of AD. Only five molecules, i.e. galantamine, donepezil, tacrine, rivastigmine, and memantine, have been approved by the USFDA for treating this disease, and even those only have symptomatic or disease-modifying effects (Agrawal, 2021).

Functional biomaterials can display desirable properties in response to any external stimulus, including changes in temperature, light, pH, a magnetic field, an electric stimulus, etc. The release of the drug to the target site or pathological circumstances, such as amyloid aggregates, tumor tissues, the site of inflammation, etc., is made possible by pH-responsive functional biomaterials. A drug delivery system, that responds to changes in pH reduces systemic exposure to AD drugs and increases drug concentration in the damaged area of the brain. It is related to the acid environment within the amyloid plaque, damaged nerve cells, or inflamed neurons, which regulates the release of the drug from this carrier or drug at the target site, causing physical or chemical changes (Agrawal, 2021). Numerous natural polymers, including chitosan, cellulose, albumin, and gelatin, exhibit behavior, that is responsive to changes in pH and temperature (Agrawal, 2021; Chatterjee, 2019). Inflamed tissues, Aβ32 aggregates, or other damaged tissues of the brain increase local temperature and scientists use this ability to create thermoresponsive biomaterials (Agrawal, 2021).

Intranasal insulin research shows, that improving insulin signaling in the brain improves memory and learning in adults with Alzheimer's disease (Gao, 2019; Dubey 2020), Gao M. et al. examined the potential of glyceryl monocaprylate–modified (GMC) chitosan on the intranasal absorption of insulin in the AD rat model. The results showed GMC-chitosan and insulin interaction at 5.8 and 6.7 pH causes insulin to be encapsulated in a polymer matrix while no/little interaction at 4.7 pH. They proved, that the tested material was a promising absorption enhancer to improve the intranasal absorption of insulin (Gao, 2019).

Zhou H. et al. designed a nanocomposite with high stability and biocompatibility by using flower-shaped hollow nano-ruthenium as a carrier. The nanocomposite contained nerve growth factor (NGF) and phase change sealing material. The blood-brain barrier was successfully penetrated by the nanocomposite due to its excellent photothermal effect under near-infrared irradiation. It can react to phase shifts in the lesion area by releasing NGF, which effectively inhibits hyperphosphorylation of tau, reduce tau aggregation, decreased oxidative stress, restores nerve damage, and maintained neuronal shape. The results showed improvement in learning and memory in the AD mice model. It indicates, that multifunctional nanocomposites may be a promising medication in the treatment of AD (Zhou, 2020).

The hydrogels, biodegradable scaffolds, carbon nanotubes, and polymeric carriers can target the medicine to the brain (Agrawal, 2021). For example, collagen can be used in wound healing, bone and cartilage repair, ophthalmology, dental applications, and peripheral nerve repair (Ucar, 2021). Foidl B.M. et al. use collagen scaffolds crosslinked with polyethyleneglycole and loaded with nerve growth factor to target the delivery of NGF to organotypic brain slices of the basal nucleus of Meyner. Collagen scaffolds enriched with small amounts of protein or drug can be easily applied directly to organotypic sections of the brain, which allows for slow, but targeted release of the protective molecule (Foidl, 2018).

PARKINSON'S DISEASE (PD)

Parkinson's disease is a neurodegenerative disease, the treatment of which is mainly centered around the supplementation of dopamine. A wide range of biomaterials ranging from biomolecules, polymers, inorganic metal, and metal oxide nanoparticles have been employed to assist in the delivery of these therapeutic agents into the brain (Krishnan, 2021). The application of biomaterials to treat Parkinson's disease is still limited, despite the constant development of materials and technology. The electroconductive hydrogel can be used as a soft material for tissue repair and regeneration of electroactive tissues, primarily nervous tissue (Xu, 2022). Xu J. et al. designed and synthesized conductive hydrogels with self-healing and anti-inflammatory properties from dialdehyde polyurethane (~36 nm) nano-crosslinker, gold nanoparticles (~15 nm), and O-carboxymethyl chitosan under physiological conditions. These hydrogels showed a stable crosslinking network, acceptable conductivity, good biodegradability, and promotion of proliferation of neural stem cells (NSCs). The results indicated an anti-inflammatory effect and the hydrogel rescued function on inflammatory NSCs. Studies have shown that injecting conductive hydrogel into the brain restores the motor function of rats with Parkinson's disease. In contrast, histological analysis showed, that injection of conductive hydrogel increased the density of tyrosine hydroxylase-positive neurons and fibers as well as reduced inflammatory responses. The proposed hydrogel may serve as a promising carrier without additional cells or drugs for the treatment of Parkinson's disease (Xu, 2022). As in PD, a nanostructured scaffold could increase the viability of the replaced cells, providing a more favorable microenvironment and promoting neurogenesis in non-neurogenetic regions. On the other hand, the nanostructured scaffold can be associated with active drugs (e.g. chemotrophic proteins) (Carradori, 2017).

GLIOBLASTOMA

Biomaterials can also be used in the treatment of glioblastoma. Natural material-based scaffolds consist of extracellular matrix-derived biomolecules, such as hyaluronic acid, collagen, fibrinogen, basement membrane extracts, and even decellularized patient tissue. The disadvantages of these materials include their origin from mammalian organisms, therefore they can contain pathogens, and also vary in soluble factors and protein concentrations. Thus, non-mammalian polymers such as alginate and chitosan can be used as they are also biocompatible with glioblastoma multiforme (GBM) cells but are not immunogenic (Stanković, 2021). Abadi B. et al. created a novel form of selenium nanoparticles functionalized with chitosan and sialic acid. They assessed the antitumor effects of obtained material on the human glioblastoma cell lines (T98 and A172). They proved that the sialic acid enhanced the stability and biological activity of biomaterial. Additionally, the nanoparticles showed better inhibitory effects on cell lines T98 (Abadi, 2023). Furthermore, Bruinsmann F.A. et al. prepared chitosan-coated simvastatin-loaded lipid-core nanocapsules (LNCSVT-chit) suitable for nose-to-brain delivery. The capsules should induce an anti-cancer effect against glioblastoma. LNCSVT-chit significantly enhanced the amount of medicine in rat brain tissue after intranasal administration. Additionally, it reduced tumor growth and malignancy in glioma-bearing rats and did not cause any toxicity (Bruinsmann, 2022). Uribe-Robles M. et al. created TiO_2 hollow nanospheres as nanocarriers for targeted drug delivery. Nanospheres were functionalized with folic acid (HT-FA) for the targeted delivery of temozolomide. HT-FA successfully delivered and internalized temozolomide to glioblastoma cancer cells with high cytotoxicity. In addition, the nanocarrier was characterized by a high loading capacity, they retain and protect the active substance for at least 48 hours. Thus, the material is a promising platform for the targeted delivery of chemotherapeutic drugs for the treatment of GBM cancer (Uribe-Robles, 2023). The materials are not only designed to deliver drugs but can also be used to increase the radiosensitivity of cancer cells. The radiosensitivity of cancer cells was also studied by Mousavi M. et al. They synthesized the SPIO@AuNP-Cisplatin-Alginate (SACA) nanocomplex, which is composed of an SPIO core, a gold shell and an alginate coating. The results indicated, that the combination of SACA and 6 MV X-rays (at the doses of 2 and 4 Gy) drastically decreased the viability of U87MG cells. Additionally, this kind of cell line treated with SACA in combination with radiation increased apoptosis, which showed that the nanocomplex effectively increases the radiosensitivity of cancer cells (Mousavi, 2023).

THERAPEUTIC BIOMATERIALS IN CARDIOLOGY

TISSUE ENGINEERING IN MYOCARDIAL REGENERATION

The major causes of death worldwide are cardiovascular diseases (CVD), which include myocardial infarction (MI) and heart failure. Cardiovascular disease is the most common non-communicable disease in the world, accounting for one-third of all fatalities with an annual death of nearly 18.6 million (Fan, 2023; Roth, 2017). MI is mainly caused by occlusion of the coronary artery, as a result of atherosclerotic and thrombotic processes, and consequent reduction of the blood flow to the heart muscle. This can lead to the death of cardiomyocytes, difficulty in the synchronous contraction of the heart, and finally life-threatening heart failure or sudden death (Pascual-Gil, 2005; Chang, 2021). Current therapies most often include surgical procedures, such as coronary bypass, balloon angioplasty, and stents. Surgical interventions are usually complemented with pharmacological treatment. However, conventional interventions can only relieve the symptoms of myocardial infarction, but cannot repair the infarcted tissue, therefore, patients after a myocardial infarction may face severe functional limitations for the rest of their lives, leading to secondary complications, that impair their quality of life (Pascual-Gil, 2015).

The greatest challenge in cardiac tissue engineering is to develop new methods for heart tissue repair and regeneration. Exploring the biological and chemical aspects of the cardiac microenvironment has been the focus of recent investigations in cardiovascular tissue engineering. Biomaterials must show high biocompatibility and biodegradability. In addition, they should reduce the local resistance of the microenvironment, promote long-term integration of transplanted cells with native tissues and serve as a carrier for the controlled release of bioactive compounds (Fan, 2023). Despite the production of various biosynthetic materials, scientists still face limitations in the form of immunological complications caused by the biodegradation of scaffolds and insufficient cell migration. Therefore, it is essential to produce natural biomaterials to aid in myocardial regeneration (Lee, 2015).

Different kinds of stem cells such as fetal cardiomyocytes, embryonic stem cells, skeletal myoblasts, crude bone marrow stem cells, hematopoietic stem cells, fibroblasts, smooth muscle cells, and induced pluripotent stem cells, have been investigated to promote myocardial repair and have shown varying degrees of success in cardiomyocyte transplantation. Several biomaterials have also been created and examined throughout the years. Injectable biomaterials, i.e. alginate, fibrin, and chitosan improve infarcted heart regeneration (Lee, 2015; Rane, 2011). Liu Z. et al. explored an injectable chitosan hydrogel for stem cell delivery into the ischemic heart. Chitosan has been commonly used as scaffolds in tissue engineering. The results of their research suggest, that chitosan hydrogel application in the ischemic myocardium could enhance the MI microenvironment. The improved MI microenvironment promoted engraftment, transplanted stem cell survival, and endogenous stem cell homing. It should be noted, that chitosan hydrogel plays relatively limited roles in controlling the MI microenvironment, mostly through reactive oxygen species (ROS) scavenging. The therapeutic effectiveness can be improved by modifying chitosan with groups like a proangiogenic peptide, a pro-adhesion peptide, and an anti-apoptotic peptide (Liu, 2012). Whereas, Wang H. et al. studied the effects of co-injection of basic fibroblast growth factor with chitosan hydrogel, which has temperature-responsive properties. The material was injected intramyocardially into the left ventricular wall of rat infarction models. The results indicate, that co-injection of basic fibroblast growth factor with temperature-responsive chitosan hydrogels enhanced the effects of basic fibroblast growth factor on arteriogenesis, ventricular remodeling, and cardiac function (Wang, 2010).

Biocompatible conductive heart patches could be a promising method of restoring cardiac function. This material supports myocardial tissue after infarction and provides sufficient electrical conductivity to transmit the heart's electrical impulses [30]. Shabankareh A.N.T. et al. fabricated electroconductive nanofibrous structures based on polyurethane/reduced graphene oxide (PU/RGO). The results of the research showed, that PU/RGO scaffolds have enhanced Young's modulus, and the ultimate tensile strength besides biocompatibility was confirmed by determining the metabolic activities of exposed endothelial and myoblast cells. PU/RGO scaffolds, even at a high amount of RGO not only did not show cell toxicity but also enhance cell proliferation. Therefore, the material could be a potential electrically conductive cardiac patch to support myocardial regeneration (Shabankareh, 2023). However, Jain A. et al. described the fabrication of nCe-decorated polycaprolactone (PCL) and PCL-gelatin blend (PCLG) nanofibers. They used an electrospinning technique for that application. The results showed, that primary cardiomyocytes cultured on nCe-decorated PCLG nanofibers showed a reduction in ROS levels when subjected to H_2O_2-induced oxidative stress. Additionally, nCe-decorated PCLG nanofibers can suppress agonist-induced cardiac hypertrophy (Jain, 2021).

BIOMATERIALS FOR HEART VALVES

Another problem in the field of cardiology is the increasing demand for replacement of heart valves. Mechanical heart valves (MHV) and biological heart valves (BHV) are the two forms of prosthetic heart valves (PHV), that were initially used in clinical settings to mimic the original features and functions of natural heart valves (Priya, 2020).

Metals like titanium or stainless steel are used to fabricate mechanical heart valves (Lam, 2012). They are generally made in a tilting-disk configuration, with one or two rigid leaflets, that rotate on hinges. The leaflets, also known as occluders, are constructed either entirely from pyrolytic carbon or graphite coated with pyrolytic carbon. These kinds of valves are generally very durable, as pyrolytic carbon is strong and quite resistant to abrasion and fatigue (Priya, 2020). Blood tends to adhere to the metal in mechanical valves, resulting in blood clots. Therefore, patients must take anticoagulant medications for the rest of their lives (Lam, 2012).

Unlike mechanical valves, natural tissue valves do not need to be treated with anticoagulants. Bovine or porcine tissues are the most often used animal sources for biological valves. Bovine valves normally last 15 to 20 years, while porcine valves typically last 8 to 15 years. On the contrary, mechanical valves made of titanium or carbon are stronger and last longer, than biological valves (usually up to 25 years). However, the body's environment is very aggressive to the material. The most common causes of bioprosthetic valve failure are calcification and tearing (Lam, 2012). Two other significant factors are fatigue and wear stress. In most cases, the body's immunological response to generated wear debris leads to biomaterial fatigue. On the other hand, transvalvular pressure (after valve closure) is considered the most stressful for mechanical valves, wear can be caused by impact and friction (Taghizadeh, 2020).

The lifespan of a replacement valve is significantly influenced by the patient's age. Due to activity and metabolism, replacement valves deplete more quickly in children and younger patients, than in elderly people. Mechanical valves are commonly implanted in patients who are 65 to 70 years old or younger; patients older than that receive bioprosthetic valves. Patients in this age group may choose any type of valve, although there is evidence, that bioprosthetic valves are a superior option since they are more likely to last the rest of the patient's life without the need to take anticoagulant drugs (Lam, 2012; Chikwe 2010).

The biopolymer materials used in the design of heart valves include silicone, polytetrafluoroethylene, polyurethane, polyvinyl alcohol or polydimethylsiloxane-polyhexamethyleneoxide-polyurethane (PDMS-PHMO-PU) (Priya, 2020). In comparison to natural biomaterials, synthetic materials are more beneficial since the synthesis process allows for precise control of their properties, including the degree of porosity, pore size, 3D structure, mechanical strength, and rate of degradation. Whereas, issues related to their biocompatibility and subsequent inflammation, thromboembolism, or thrombosis can cause limitations in their application. In addition, special attention should be paid to the toxicity of synthetic materials and biodegradable materials (Lam, 2012).

Lancellotti P. et al. produced a drug-releasing multilayer coating adherent to mechanical valves. Their coating consisted of ticagrelor- and minocycline-releasing cross-linked nanogels covalently linked to polyethylene glycol. They assessed the hydrodynamic performance, durability, and hemocompatibility of coated valves. As opposed to noncoated valves, valve thrombosis was effectively reduced, when coated valves were implanted in nonanticoagulated pigs for a month (Lancellotti, 2023). Dehghani F. et al. also worked on improving hemocompatibility. They modified nanocomposites based on polyurethane – carbon nanotubes with heparin for application in heart valves. Then the nanocomposite was subjected to sulfuric acid and nitric acid oxidization. Their results indicated, that the obtained biomaterial reduced platelet adhesion and accumulation on the surface (Dehghani, 2022). However, Zheng C. et al. proposed a glutaraldehyde-free crosslinking method. The porcine pericardium was treated with 2-isocyanatoethyl methacrylate to introduce methacrylate groups and subsequently copolymerized with crosslinker, poly(ethylene glycol) dimethacrylate (PEGDA), to prepare a PEGDA polymer crosslinked porcine pericardium. The results showed, that the cytocompatibility and stability of obtained material were significantly improved. In addition, anti-thrombotic and anti-calc properties have been confirmed (Zheng, 2022).

Nanodiamonds are used in dental care, bio-imaging, and the creation of matrix composites for drug administration because of their great biocompatibility and outstanding mechanical properties (Chernysheva, 2023). Chernysheva M.G. et al. prepared nanodiamond-chitosan on the surface of the collagen tissue

crosslinked by glutaraldehyde. They compared the biostability and mechanical properties of the coatings with positively and negatively charged nanodiamonds. The results showed, that layer-by-layer applied nanodiamond and chitosan films improved the mechanical properties of the bovine pericardium. This was noticeable for both positively and negatively charged nanodiamonds (Chernysheva, 2023). Whereas, Tang X.S. et al. fabricated molybdenum doped diamond-like carbon (Mo-DLC) coatings, which were deposited by closed field unbalanced magnetron sputtering. The findings demonstrated, that a Mo-DLC coating with a low molybdenum concentration was a protective coating with good wear resistance at an ambient temperature of 500°C, reduced residual stress, and increased cohesive strength. The results indicated that there was significantly less thrombus on the Mo-DLC nanocomposite coatings, than there was on the pyrolytic carbon films (Tang, 2014).

BIOMATERIALS FOR STENTS

The repeated narrowing of the dilated segment of a coronary artery is known as restenosis. An artery's diameter narrowing by at least 50% on a subsequent coronary angiography is known as angiographic restenosis. A stent is inserted into the coronary artery in about 80% of all percutaneous coronary procedures. Each year, about 4.0 million of these procedures are carried done globally (Sareło, 2023; Pleva, 2018). Despite a significant reduction in the incidence of this phenomenon in patients, in-stent restenosis (ISR) resulting from neointimal hyperplasia is still a real threat and affects the success of the procedure (Pleva, 2018).

Materials used in prosthetic heart valves and vascular stents must minimize thrombosis, which is responsible for the local inflammatory response. It is important to reduce the adhesion of platelets and macrophages to the scaffold material and to minimize the risk of thrombosis due to the direct contact of the material with the patient's blood (Peng, 2022). Despite having excellent mechanical properties, the metals used to fabricate stents nevertheless have certain disadvantages, including restenosis, thrombosis associated with stents, and occlusion. The primary side effects of stents, such as thrombosis and allergic reactions, are caused by the release of metal ions and should be avoided (Malisz, 2023). Drug-eluting stents (DES) are the most popular type of stents used for this purpose. Antiproliferative drugs (such as sirolimus, paclitaxel, and everolimus) are released locally by DES, preventing excessive neointimal hyperplasia following stent placement and decreasing the incidence of ISR (Pleva, 2018). Scientists are still developing biomaterials and using different substances to create the most compatible stent.

Sareło P. et al. proposed a polydopamine-based-coating functionalized with an anti-inflammatory interleukin. Polydopamine has found application in the design of drug delivery systems due to its highly desirable properties such as the ability to be loaded with drugs to their controlled release and excellent photothermal conversion efficiency. The results of *in vitro* studies showed the promotion of endothelialization in the initial stage after implantation. Although, they confirmed the immunological activity of the coating by assessing the changes in THP-1 differentiation, which indicated that the binding procedure does not impair the biological properties of the interleukin. It can be said that the proposed anti-inflammatory coating can reduce the probability of restenosis to a minimum (Sareło, 2023). Whereas, Saadatlou G.A. et al. prepared a tetra-functional coating, which contains poly(2-ethyl-2-oxazoline)-co-polyethyleneimine (PEOX-co-PEI) stabilized silver nanoparticles and heparin. The coatings were deposited on NiTi alloy and 316 L stainless steel substrates via a layer-by-layer technique. The results indicated, that the material shows anticorrosive, antibacterial, biocompatible, and anticoagulant properties (Saadatlou, 2023). However, Hua J. et al. proposed a silk fibroin/chitosan-based (SF/CS/Cu) biopolymer coating incorporating copper ions. The results indicate, that the coating allowed for the migration and proliferation of endothelial cells on the cardiovascular stent surface. In addition, the NO-generating ability of SF/CS/Cu coatings may be used in the treatment of cardiovascular diseases (Hua, 2023). The other idea was presented by Wang B. et al., who created the bioactive hydrogel coating, which was based on chitosan, catechol groups, and copper ions. The findings of the experiments showed, that it is possible to accurately regulate the creation of the chitosan hydrogel coating using electrochemical deposition and functionalize it with catechol groups to further enhance its biological properties. Additionally, it has been possible to obtain accelerated NO-generation activity and *in vitro* cell biocompatibility. This suggests that biomimetic hydrogel coating may be a potential material for vascular engineering (e.g., coronary stent) and other biomedical devices (Wang, 2021).

The stent's surface can be covered with biocompatible and protective materials such as diamond-like carbon films. Many research indicates that DLC demonstrates high biocompatibility, haemocomability, strong adhesion to the substrate and reduction of platelet adhesion, in addition, this type of coating does not cause any inflammatory response or is toxic to cells (Malisz, 2023; Okpalugo, 2004). Castllino M. demonstrated that DLC coatings, deposited by physical vapour deposition, promote endothelization of coronary stent devices. DLC film coating enhanced haemocomability and biocompatibility and promotes excellent early vascular healing of the stent, besides, extremely thin strut thickness reduces the amount of late neointima and also the risk of late restenosis (Castellino, 2013). Additionally, doping with F enhances the anti-thrombogenic properties of DLC (Malisz, 2023; Saito, 2005).

CONCLUSION

Biomaterials are a group of natural and synthetic substances used to create scaffolds, designed to support the differentiation and proliferation of stem cells and, as a result, regenerate damaged tissue, but also support, and replace a given tissue or organ or targeted treatment of tissues/organs. Materials, that are implanted in the human body meet very specific requirements, i.e. they must not be toxic or immunogenic, they must be biocompatible, and have appropriate mechanical properties, support cell proliferation, or degrade without causing inflammatory reactions. There are many expectations. Demand is growing. Despite significant progress in the field of biomaterials for use in neurology and cardiology, scientists still have many goals to achieve. Improving existing technologies, drug delivery systems or interactions of the material with the body is essential.

References

Sternberg K. **Current requirements for polymeric biomaterials in otolaryngology**. GMS Curr. Top. Otorhinolaryngol. Head Neck Surg. 2009; 8: Doc11.

Lally C., Joyce K., Pandit A**. Biomaterials enhancing performance of cell and nucleic-acid therapies: An opportunity in the brain**. Biomater. Biosyst. 2022; 5: 100036.

Ucar B. **Natural biomaterials in brain repair: A focus on collagen**. Neurochem. Int. 2021; 146:105033.

Vasu S., Zhou J., Chen J., Johnston P.V., Kim D.-H. **Biomaterials-based Approaches for Cardiac Regeneration**. Korean Circ. J. 2021; 51(12): 943-60.

Fan C., He J., Xu S., Yan J., L. Jin, Dai J., et al. **Advances in biomaterial-based cardiac organoids.** Biomater. Adv. 2023;153: 213502.

Akhtar A., Farzam Rad V., Moradi A.-R., Yar M., Bazzar M. **Emerging polymeric biomaterials and manufacturing-based tissue engineering approaches for neuro regeneration-A critical review on recent effective approaches**. Smart Mater. Med. 2023; 4: 337-355.

Agrawal M., Prathyusha E., Ahmed H., Dubey S.K., Kesharwani P., Singhvi G., et al. **Biomaterials in treatment of Alzheimer's disease.** Neurochem. Int. 2021; 145: 105008.

Hampel H., Vergallo A., Caraci F., Cuello A.C., Lemercier P., Vellas B., et al. **Future avenues for Alzheimer's disease detection and therapy: liquid biopsy, intracellular signaling modulation, systems pharmacology drug discovery**. Neuropharmacology. 2021; 185: 108081.

Azargoonjahromi A. **Dual role of nitric oxide in Alzheimer's disease**. Nitric Oxide. 2023; 134-135: 23-37.

Chatterjee S., Chi-leung Hui P**. Review of Stimuli-Responsive Polymers in Drug Delivery and Textile Application.** Molecules. 2019; 24(14): 2547

Gao M, Sun Y., Kou Y., Shen X., Huo Y., Liu C., et al. **Effect of Glyceryl Monocaprylate–Modified Chitosan on the Intranasal Absorption of Insulin in Rats**. J. Pharm. Sci. 2019; 108(11): 3623-29.

Dubey S.K., Lakshmi K.K., Krishna K.V., Agrawal M., Singhvi G., Saha R.N, et al. **Insulin mediated novel therapies for the treatment of Alzheimer's disease**. Life Sci. 2020; 249: 117540.

Zhou H., Gong Y., Liu Y., Huang A., Zhu X., Liu J., et al. **Intelligently thermoresponsive flower-like hollow nano-ruthenium system for sustained release of nerve growth factor to inhibit hyperphosphorylation of tau and neuronal damage for the treatment of Alzheimer's disease**. Biomaterials. 2020; 237: 119822.

Foidl B.M., Ucar B., Schwarz A., Rebelo A.L., Pandit A., Humpel C. **Nerve growth factor released from collagen scaffolds protects axotomized cholinergic neurons of the basal nucleus of Meynert in organotypic brain slices**. J. Neurosci. Methods. 2018; 295: 77-86.

Krishnan U.M. **Biomaterials in the treatment of Parkinson's disease**. Neurochem. Int. 2021; 145: 105003.

Xu J., Tai C.-H., Chen T.-Y, Hsu S. **An anti-inflammatory electroconductive hydrogel with self-healing property for the treatment of Parkinson's disease.** Chem. Eng. J. 2022; 446(3): 137180.

Carradori D., Eyer J., Saulnier P., Préat V., des Rieux A. **The therapeutic contribution of nanomedicine to treat neurodegenerative diseases via neural stem cell differentiation**. Biomaterials. 2017; 123: 77-91.

Stanković T., Ranđelović T., Dragoj M., Stojković Burić S., Fernández L., Ochoa I., et al**. In vitro biomimetic models for glioblastoma-a promising tool for drug response studies**. Drug Resist. Updat. 2021; 55: 100753.

Abadi B., Khazaeli P., Forootanfar H., Ranjbar M., Ahmadi-Zeidabadi M., Nokhodchi A., et al. **Chitosan-sialic acid nanoparticles of selenium: Statistical optimization of production, characterization, and assessment of cytotoxic effects against two human glioblastoma cell lines**. Int. J. Pharm. 2023; 637: 122884.

Bruinsmann F.A., de Cristo Soares Alves A., de Fraga Dias A., Lopes Silva L.F., Visioli F., Raffin Pohlmann A., et al. **Nose-to-brain delivery of simvastatin mediated by chitosan-coated lipid-core nanocapsules allows for the treatment of glioblastoma in vivo**. Int. J. Pharm. 2022; 616: 121563.

Uribe-Robles M., Ortiz-Islas E., Rodriguez-Perez E., Valverde F.F., Lim T., Martinez-Morales A.A. **Targeted delivery of temozolomide by nanocarriers based on folic acid-hollow TiO2 -nanospheres for the treatment of glioblastoma**. Biomater. Adv. 2023; 151: 213442.

Mousavi M., Koosha F., Neshastehriz A. **Chemo-radiation therapy of U87-MG glioblastoma cells using SPIO@AuNP-Cisplatin-Alginate nanocomplex**. Heliyon. 2023; 9(3): e13847.

Roth G.A., Johnson C., Abajobir A., Abd-Allah F., Abera S.F., Abyu G., et al. **Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 201**. J. Am. Coll. Cardiol. 2017; 70(1): 1-25.

Pascual-Gil S., Garbayo E., Díaz-Herráez P., Prosper F., Blanco-Prieto M.J. **Heart regeneration after myocardial infarction using synthetic biomaterials**. J. Control. Release. 2015; 203: 23-38.

Chang T., Liu C., Lu K., Wu Y., Xu M., Yu Q., et al. **Biomaterials based cardiac patches for the treatment of myocardial infarction**. J. Mater. Sci. Technol. 2021; 94: 77–89.

Lee K.M, Kim H., Nemeno J.G., Yang W., Yoon J., Lee S. et al. **Natural Cardiac Extracellular Matrix Sheet as a Biomaterial for Cardiomyocyte Transplantation**. Transplant Proc. 2015; 47(3): 751-6.

Rane A.A., Christman K.L. **Biomaterials for the Treatment of Myocardial Infarction: A 5-Year Update**. J. Am. Coll. Cardiol. 2011; 58(25): 2615-29.

Liu Z. , Wang H., Wang Y., Lin Q., Yao A., Cao F., et al. **The influence of chitosan hydrogel on stem cell engraftment, survival and homing in the ischemic myocardial microenvironment**. Biomaterials. 2012; 33(11): 3093-3106.

Wang H., Zhang X., Li Y., Ma Y., Zhang Y., Liu Z., et al. **Improved myocardial performance in infarcted rat heart by co-injection of basic fibroblast growth factor with temperature-responsive Chitosan hydrogel**. J. Hear. Lung Transplant. 2010; 29(8): 881-7.

Shabankareh A.N.T., Samadi Pakchin P., Hasany M., Ghanbari H. **Development of a new electroconductive nanofibrous cardiac patch based on polyurethane-reduced graphene oxide nanocomposite scaffolds**. Mater. Chem. Phys. 2023; 305: 127961.

Jain A., Behera M., Mahapatra C., Sundaresan N.R., Chatterjee K. **Nanostructured polymer scaffold decorated with cerium oxide nanoparticles toward engineering an antioxidant and anti-hypertrophic cardiac patch**. Mater. Sci. Eng. C. 2021; 118: 111416.

Priya C.H., Divya M., Ramachandran B., **Recent investigation on biomaterial based tissue engineered heart valve (TEHV)**. Mater. Today Proc. 2020; 33(7): 4467-78.

Lam M.T., Wu J.C., **Biomaterial applications in cardiovascular tissue repair and regeneration**. Expert Rev. Cardiovasc. Ther. 2012; 10(8): 1039-49.

Taghizadeh B., Ghavami L., Derakhshankhah H., Zangene E. **Biomaterials in Valvular Heart Diseases**. Front. Bioeng. Biotechnol. 2020; 8: 1-20.

Chikwe J., Filsoufi F., Carpentier A.F. **Prosthetic valve selection for middle-aged patients with aortic stenosis**. Nat. Rev. Cardiol. 2010; 7(12): 711-9.

Lancellotti P., Aqil A., Musumeci L., Jacques N., Ditkowski B., Debuisson M., et al. **Bioactive surface coating for preventing mechanical heart valve thrombosis**. J. Thromb. Haemost. 2023; 1538-7836(23): 00416-6

Dehghani F., Khorasani M.T., Movahedi M. **Fabrication of polyurethane – Heparinized carbon nanotubes composite for heart valves application**. Mater. Chem. Phys. 2022, 280: 125819.

Zheng C., Ding K., Huang X., Yang L., Lei Y., Wang Y. **A bioprosthetic heart valve prepared by copolymerization of 2-isocyanatoethyl methacrylate modified pericardium and functional monomer**. Compos. Part B Eng. 2022; 238: 109922.

Chernysheva M.G., Chaschin I.S., Badun G.A., Vasil'ev V.G., Mikheev I. V, Shen T., et al. **Novel nanodiamond coatings for durable xenogenic heart valve prostheses: Mechanical properties and in vivo stability**. Colloids Surfaces A Physicochem. Eng. Asp. 2023; 656(A): 130373.

Tang X.S., Wang H.J., Feng L., Shao L.X., Zou C.W. **Mo doped DLC nanocomposite coatings with improved mechanical and blood compatibility properties**. Appl. Surf. Sci. 2014; 311(30): 758-62.

Sareło P., Sobieszczańska B., Wysokińska E., Gąsior-Głogowska M., Kałas W., Podbielska H., et al. **In vitro examinations of the anti-inflammatory interleukin functionalized polydopamine based biomaterial as a potential coating for cardiovascular stents**. Biocybern. Biomed. Eng. 2023; 43(1); 369-85.

Pleva L., Kukla P., Hlinomaz O. **Treatment of coronary in-stent restenosis: a systematic review**. J. Geriatr. Cardiol. 2018; 15(2): 173–84.

Peng Y., Peng J., Wang Z., Xiao Y., Qiu X. **Diamond-like Carbon Coatings in the Biomedical Field: Properties, Applications and Future Development**. Coatings. 2022; 12(8): 1088.

Malisz K., Świeczko-Żurek B., Sionkowska A. **Preparation and Characterization of Diamond-like Carbon Coatings for Biomedical Application - A Review**. Materials. 2023; 16(9): 3420.

Saadatlou G.A., Ijaz A., Sipahioğlu D., Surme S., Kavakli I.H., Gurpinar Y., et al. **Tetra-functional multilayer coatings for cardiovascular stent materials**. Colloids Surfaces A Physicochem. Eng. Asp. 2023; 670: 131571.

Hua J., Yang H., Wang B., Dai Y., Li X., Yan K., et al. **Silk fibroin/chitosan coating with tunable catalytic nitric oxide generation for surface functionalization of cardiovascular stents**. Int. J. Biol. Macromol. 2023; 228: 261-72.

Wang B., Hua J., You R., Yan K., Ma L. **Electrochemically deposition of catechol-chitosan hydrogel coating on coronary stent with robust copper ions immobilization capability and improved interfacial biological activity**. Int. J. Biol. Macromol. 2021; 181: 435–43.

Okpalugo T.I.T., Ogwu A.A., Maguire P.D., McLaughlin J.A.D., Hirst D.G. **In-vitro blood compatibility of a-C:H:Si and a-C:H thin films**. Diam. Relat. Mater. 2004; 13(4-8): 1088–92.

Castellino M., Stolojan V., Virga A., Rovere M., Cabiale K., Galloni M.R., et al. **Chemico-physical characterisation and in vivo biocompatibility assessment of DLC-coated coronary stents**. Anal. Bioanal. Chem. 2013; 405(1): 321–9.

Saito T., Hasebe T., Yohena S., Matsuoka Y., Kamijo A., Takahashi K., et al. Antithrombogenicity of fluorinated diamond-like carbon films**. Diam. Relat. Mater. 2005; 14(3-7): 1116-19.**

Review and Research on Cancer Treatment
Volume 9, Issue 1 (2023)

Beneficial properties of snail slime, its use in medicine and cosmetology – a review of current research

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ABSTRACT

The purpose of this chapter is to present the mucus produced by snail in terms of its practical application in modern cosmetology and medicine. Due to the development of the pharmaceutical industry, laboratories around the world carry out research on the acquisition of natural, non artificially synthesized substances that can be used as cosmetics or medicines. The side effects of using synthetic pharmaceuticals are chronic allergic and allergic reactions. Natural substances produced by animals, including e.g. by snails may in future be a cheap, available and alternative raw material used in the cosmetics industry and in medicine (Domínguez-Martín, 2020; Cristiano, 2022).

Due to its antibacterial, regenerative, antioxidant and rejuvenating properties, snail slime is used in cosmetology and dermatology. It is also used in medicine in anti-cancer therapy. Studies have shown antisuppressive effects in skin cancers (melanoma), and also in breast, lung, colon and bladder cancers. Studies have confirmed the effectiveness of snail mucus in allergic skin diseases, e.g. atopic dermatitis, in some respiratory diseases and unusual allergic and asthmatic diseases. It has also found application in the treatment of stomach ulcers and helps in lowering sugar levels in diabetes. It is recommended for extensive burns in the natural stimulation of wound healing. There are also mentions that the copper peptides contained in the snail slime may affect hair growth by stimulating the progenitor stem cells.

The effect of snail mucus in humans on skin viruses, especially the herpes virus HSV, belonging to the family of Herpesviridae, is special, and perhaps, being an affinity for the mucous membranes of the lung epithelium, it may in the future be a breakthrough in the succor of treatment of the SARS-CoV-2 virus. These reports may prompt scientists and biotechnologists to undertake further research (Pons, 1998; Pons, 1999).

The healing effect on human skin was known to the shamans of the indigenous peoples of South America and Africa. Hippocrates also applied it to ulcers and wounds and recommended the use of crushed snails on inflamed skin. Also in the Middle Ages, the health-promoting (immunostimulating) properties of mucus were appreciated.

The properties of snail slime were "discovered" by modern medicine in the 20th century. In Chilean family, the members which had a contact with snail slime, had healthy, well-groomed skin on their hands, despite of the hard work. Abrasions and cuts healed quickly without leaving scars. Analyzes have shown that the mucus extract, containing, among others, Cryptosine has valuable properties for human skin. They began to be used in care and cosmetic preparations.

The effect of cosmetics and drugs with snail slime is diverse and multidirectional. It can be used as a natural raw material in the pharmaceutical industry – an alternative to substances obtained by chemical synthesis. Its acquisition is relatively cheap and has a humane and ecological character. The strong regenerative properties of the mucus can be used on a larger scale in the future for the production of preparations with high care, regenerative and therapeutic values, and at the same time devoid of allergenic properties. The use of the potential hidden in the snail slime is a reference to the practices of well-known etnopharmacies and imitation of nature.

INTRODUCTION

Mucilages are a group of mixtures of substances of a polysaccharide nature. They have been used in medicine as coating, protective and softening agents. In the presence of water, they form sticky colloids. Mucilages are used in inflammation of the gastrointestinal tract and respiratory tract, as coating and protective agents.

Mucilages and raw materials containing them are also used as laxatives. Absorbing water from the lumen of the gastrointestinal tract, they gradually swell, which causes stretching of the intestinal walls and stimulation of their peristalsis by reflex. Slightly less often they are applied externally to the surface of the skin (McShane, 2021).

They occur most often in algae, some fungi and tissues or seeds of plants, mainly from the family: Malvacae Juss., Linacae DC. ex Gray, Fabacae Lindl. and Rosaceae Juss. An interesting group of mucus-containing organisms are slime molds (Myxomycota, Myxomycetes) also called slime molds (Eumycetozoa). These are eukaryotic organisms on the border of plants, fungi, protozoa and animals, belonging to the supergroup Amoebozoa (Stephenson, 1994; Royas, 2021).

Typical plant mucous raw materials include, a.o.: agar which is a component of Rodophyta red algae mucus, marshmallow root – *Althaceae radix*, comfrey root – *Symphyti radix*, marshmallow leaf – *Althaeae folium*, coltsfoot leaf – *Farfarae folium*, fenugreek seed – *Foenugraesi semen*, flax seed – *Lini semen*, psyllium

seed – *Psylli semen*, forest mallow flower – *Malvae sylvestris flos*, mullein flower – *Verbasci flos* and Icelandic lichen – *Lichen islandicus* (Muller, 2001; Fabricant, 2001; Dias, 2012; Kohlmüzner, 2013).

The properties of mucilages of plant origin have been well known and have found wide application. However, this cannot be said about mucilages of animal origin. They mainly recognize lubricants in the processes of sexual reproduction. In other animals belonging to both invertebrates and vertebrates, mucus covering the surface of the body protects against water loss and drying out. Mucin contained in mucus is part of bile, digestive juices, occurs in saliva and also covers the mucous membrane in the respiratory system. The production of mucus by some animals makes it easier for them to move (Quevauviller, 1953; McShane, 2012; Nouthan, 2021).

GENERAL CHARACTERISTICS OF SNAILS

Molluscs (Molusca) is a type of invertebrate animal that currently numbers more than 125,000 species. They are one of the most numerous types of animals found in the world, second only to arthropods Arthoropoda. They owe their name to the fact that their bodies are soft, without a skeleton (molluscus from Latin means soft), only some of them have an external skeleton, in the form of shells. They live both in the seas, fresh waters as well as on the land. They form an extremely diverse group of animals in terms of appearance and size. The study of mollusks is carried out by a science called malacology (malacozoology). The systematic division of mollusks is based on differences in morphological characteristics. Eight classes have been recognized: Monoplacophora, Polyplacophora, Caudofoveata, Aplacophora, Scaphopoda, Gastropoda, Bivalvia and Cephalopoda. Of the above, the last three are the most characteristic: bivalves, cephalopods and snails (Grabda, 1983; Jura, 2007).

Gastropods (Gastropoda, from Greek γαστήρ – belly, πούς – leg, foot) are one of the most numerous and diverse phylums of molluscs. Due to the development of respiratory organs, snails are divided into lung (terrestrial and freshwater forms), forebronchial (they occur mainly in the sea and less often on the land) and hindbronchi (live exclusively in the sea). There is also a division into naked and shelled snails, but this is not a systematic division (Błaszak, 2009; Jura, 1983; Jura, 2007; Moore, 2006).

Snails are characterized by asymmetry. The snail is made of a head, a strongly muscled leg and a visceral sac, covered with a fold called mantle, which contains most of the internal organs, in most molluscs it also produces a hard external skeleton in the form of shells. On the head there is a mouth and sense organs, among others antennae and eyes. There are three pairs of antennae. The first, larger pair with eyes, located at the top of the head, allows you to distinguish light from darkness. The second, smaller, serves as an organ of touch. The third is transformed into labial lobes, also called cheek. In the mouth there is a fleshy tongue covered with a grater (radula), with teeth arranged in rows for grinding and grinding food. In moments of danger, the snail pulls the antennae, head or whole body deep into the shell. It is covered with ciliated epidermis, except for the part covered by a shell. In the epidermis of the leg there are numerous, different types of mucous glands producing mucous substances of different composition. There may be a single, large mucous gland on the front and back of the leg. The snails moves thanks to a strongly muscled leg, it is a locomotor organ. By creeping, it simultaneously produces large amounts of friction-reducing mucus (Jura, 1983; Lai, 2010).

There are different types of mucous glands in the snails: i) type A glands secreting proteins, ii) type B glands secreting calcium ions, iii) type C glands secreting dye/pigment, iv) type D glands secreting lipids/fats, v) type E glands secreting other substances (Arcardi, 1967; Cook, 1983; Martin, 1986; Gural-Sverlova, 2011; Wondrak, 2012; van Byern, 2018; Yamaguchi, 2000; Greistorfer, 2020).

Snail mucus performs various functions and exhibits various properties: 1) lubricating and softening – reduces friction when moving, 2) adhesive facilitates attachment to various surfaces, 3) moisturizing – protects against water loss, 4) protective – makes snails unattractive to potential predators, 5) repairing – facilitating the healing of cuts and wounds and prevents infections thanks to bioactive compounds (Mair, 2002; Pawlicki, 2004; Brieva, 2008; Lai, 2010; Becker, 2012; Newton, 2012; Newar, 2015; Li, 2017; Ahmad, 2018).

The composition of the snail mucus varies depending on the species and its role, the path that the snails is currently moving or the need for adhesion. It consists of more than 90% water. The remainder of the mucus consists of a mixture of proteoglycans, glycosaminoglycans, glycoprotein enzymes, hyaluronic acid, copper

peptides (GKH-Cu peptides), antimicrobial peptides and metal ions, a.o. iron, copper, zinc and manganese (Braun, 2013; Greistorfer, 2017; Greistorfer, 2023).

Ones of the most characteristic are: several mucins and also limozine and cryptosine. Snail slime also contains allantoin, collagen, elastin and glycolic acid (Smith, 1999).

The visible wet trace left by a moving, slowly creeping snail solidifies into a transparent or pearl "shell" – it is limozine, which is useless for humans, because it does not contain valuable substances. It is sticky and transparent, although visible to the naked eye. Its task is to reduce friction during the movement of the snail. It is also secreted for protective purposes – in situations of danger and stress and in changing environmental conditions, e.g. in summer, during drought or in winter, when the snail goes into hibernation – it creates a thin film closing the shell called an epiphragm, protecting the snails from drying out or cold. Less visible, thick and foamy mucus – is a cryptosine that protects the delicate body of the snail and regenerates it when it is damaged while moving. It also serves to thermally regulate and hydrate the body of the snail (Adikwu, 2005). That's why it's find application in cosmetology and medicine.

So far, appreciating the taste of snail meat containing a lot of valuable ingredients a.o. microelements (e.g. selenium), snail farms have been carried out, mainly for consumption purposes. The species most commonly consumed by humans include representatives of the family Helicidae, the genus *Helix* and the family Achatinidae, the genus *Achatina* (Ligaszewski, 2005; Szkucik, 2011; Paszkiewicz, 2014).

Each species has its own commercial name: Escargots de Bourgogne for *Helix pomatia*, Escargots Petit-Gris and Gros-Gris for *Helix aspersa*. In Polish, the edible land grey snail (*Helix aspersa*) is bred in two subspecies: the Western European grey snail (*Helix aspersa aspersa, Helix aspersa* Müller) and the North African large grey snail (*Helix aspersa* Maxima). Closely related to them is the naturally occurring grape snail *Helix pomatia*. Its taste qualities are slightly inferior to the other two species. In Poland, this species is also known under another name, accurately reflecting its appearance – small gray snail (petit gris). It is approved for breeding in special in the so-called Eco Snail Farm (Ciszewski, 2012; Skalmowski, 2020).

Natural breeding gives more valuable products. Organic farming of snails should comply with the requirements of the species, provide animals with freedom of movement and comfort – as close as possible to natural living conditions. It is very important to get snails ecological methods, the use of chemicals in cultivation is avoided, they should be microbiologically clean, free from parasites and heavy metals in their tissues (Niewiadomska, 1981; Menta, 2001; Morozińska-Gogol, 2016).

PROPERTIES OF SNAIL MUCUS

Snail mucus is widely used in medicine a.o. in 1) antibacterial, antifungal and even antiviral effects, 2) action in burns and wound healing, 3) action in stomach ulcers, 4) action in atopic dermatitis, 5) action in chronic bronchitis, 6) action in bone fractures and regeneration, rheumatic diseases, toothache, 7) action in the treatment of cancer: skin (melanoma), breast, lung, colon, bladder cancer, 8) actoxidative effect in skin aging processes, 9) action in the treatment of skin lesions, a.o. wrinkles (water pulling), scars, 10) anti-inflammatory and antibacterial effect of mucus in complexes with precious metals (colloidal nanosilver and nanogold) and zinc (Ahmad, 2018; Cilia, 2018; Mane, 2021).

Due to its antibacterial and regenerative properties, snail mucus is widely used in cosmetology and dermatology. It is also used in medicie in some respiratory diseases and atypical allergic and asthmatic diseases (Pons, 1998; Pons, 1999). Studies have confirmed the effectiveness in allergic skin diseases a.o. atopic dermatitis (Min-Jee, 2010). It has also been used in the treatment of stomach ulcers (Mu, 2008; Amah, 2019; Gugliandolo, 2021) and it helps in lowering sugar levels in diabetes (Agu, 2018). There are also scientific reports about its effectiveness in cancer therapy (Antonova, 2014). Studies have shown antisuppressive effects mainly in skin cancers (melanoma), as well as in breast, lung, bladder and colon cancer (Dwek, 2001; Laack, 2002; Lescar, 2007; Boyanova, 2013; Gesheva, 2014; Ekobon, 2016; Matusiewicz, 2018; Ellijimi, 2018; Dolashki, 2019).

Antimicrobial activity is ensured by the specific immunological properties of the snail (survival in environmental conditions – produced by evolutionary changes): antibacterial, antifungal and antiviral are shown especially by water snails. The effect of snail mucus in humans on skin viruses, especially the herpes virus HSV, belonging to the *Herpes* viridae family, is particularly special, and perhaps it may be of breakthrough importance in the succor of treatment of the SARS-CoV-2 virus – constituting an affinity for the mucous

membranes of the pulmonary epithelium. These reports may prompt scientists and biotechnologists to undertake further research (Pons, 1998; Pons, 1999).

Numerous scientific reports indicate the cytotoxic properties of snail mucus in relation to various cell lines especially from fibroblasts, including cancerous epithelial origin. Scientific research has shown that snail slime *Helix aspersa maxima* has antitumor activity against human cells melanoma malignum, while *Cornu aspersum* snail lyophilisate significantly reduces the survival rate of Caco-2 colon cancer cells. In addition, hemocyanins obtained from water snails (*Rapana venosa*) and land snails (*Cornu aspersum* and *Helix lusitanicus*) are immunostimulants with antimicrobial and antitumor properties, cytotoxic to bladder cancer cells of the T-24 line (Boyanova, 2013).

Further comprehensive studies showed that also the hemolymph of aqueous snails *Rapana thomasina* and terrestrial snails *Helix pomatia* has antitumor activity against colorectal cancer cells of the C-26 line, and the mucus of land snails *Achatina fulica* is cytotoxic to breast cancer cells of the MCF-7 line and renal epithelial cells of the Vero-fibroblast line (Dwek, 2001; Ekobon, 2016).

HISTORICAL DATA

Snail mucus does not show potential allergic reactions in humans, and due to its distant evolutionary relationship, it gives a lower risk of cross-reactions. Its healing effect on human skin was known to shamans of indigenous peoples of South America and Africa. Hippocrates also applied it to ulcers and wounds and recommended it the use of crushed snails on inflamed skin. Also in the Middle Ages, the health-promoting (immunostimulating) properties of mucus were appreciated (Gomot, 1998; Ikejiuba, 2005; Ciszewski, 2012; Ahmad, 2018).

The properties of snail slime were once again "discovered" by modern medicine in the 20th century and it was by chance. It was noticed that members of the Chilean Bascuñàn family, breeding *Helix aspersa* Muller snails for food purposes, had healthy, well-groomed skin on their hands every day, despite the fact that for years it was exposed to abrasions and cuts, dried by the scorching sun. It was found that numerous abrasions and cuts healed quickly without leaving scars. Fernando Bascuñàn Ygualt, a doctor and son of one of the breeders, decided to investigate. The analyzes initiated by him proved that mucus extract has valuable properties for human skin. This led to the discovery of cryptosine, which began to be used in care and cosmetic preparations. It has been proven that the characteristic ability of snail mucus to constantly regenerate its body shells also applies to human skin. In addition to cryptosine, snail slime contains ingredients that are found in synthetic form in most cosmetics. Therefore, it can be a better – natural alternative to artificially produced products. Since then, products based on snail slime have gained great popularity. Currently, snail slime extract is listed in the International Nomenclature of Cosmetic Ingredients (Yongeun, 2022).

SLIME OBTAINING METHOD

Methods of obtaining snail mucus raise ethical doubts. In the past, very drastic and inhumane techniques were used, such as: treatment with alcohol or vinegar, ozonation, rapid centrifugation and cooling. Currently, the least traumatic method is considered to be very gentle, slow spinning. The method of stimulation with a highly diluted solution of citirc acid is also used (Das, 2022; Ricci, 2023).

The producers reserve the patent rights regarding the details of the methodology of obtaining, purifying and treating the snail slime. There are also non-ethical reasons for this. A stressed snail, in defense against a threat, may secrete harmful substances, e.g. limnousine (Adikwu, 2005). The entire process is carefully monitored. Farms where mainly cryptosine and also other mucins are obtained imitate the natural living environment of snails. After collection, the mucus intended for the production of cosmetics is filtered, standardized and processed, which ensures its appropriate purity. It comes from healthy, free from parasites and microorganisms, specially selected specimens (Niewiadomska, 1981; Morozińska-Gogol, 2016; Lim, 2020).

SNAIL MUCIN

Snail mucins are complex, high-molecular glycoproteins that contain: polysaccharides, hyaluronic acid, elastin, collagen, allantoin, as well as vitamins C and E, A, B6 and B12. The rich composition has become the reason for the popularity of snail mucin in the cosmetics industry. Mucins have the ability to adsorb water, hence they prevent the formation of wrinkles. These substances relieve inflammation and allergic

conditions, counteract the symptoms skin aging, exfoliate dead skin cells. They help heal wounds and reduce scars, reduce wrinkles and reduce stretch marks (Ikejiuba, 2005; Sanchez, 2006; Brieva, 2008; Gabriel, 2011; El Mubaraka, 2013; Dolashka, 2015; Swapna, 2015; Liu, 2017; McDermott, 2021; Khrokalo, 2022; Yongern, 2022).

HYALURONIC, GLYCOLIC ACIDS AND OTHERS

The hyaluronic acid contained in mucin is credited with moisturizing properties that support the skin barrier and help retain moisture. Glycolic acid helps stimulate collagen production, which reduces fine lines and wrinkles, and helps give skin a radiant, youthful glow. It also contains zinc, which has an anti-inflammatory effect, and allantoin, which soothes irritations. Thus, skin can be expected to become softer, more hydrated and radiant with consistent use of snail mucin.

COPPER PEPTIDES

Copper peptide-1 (GHK-Cu) is a naturally occurring peptide complex composed of a copper molecule and the amino acids glycyl-L-histidyl-L-lysine. GHK-Cu tripeptide has a strong affinity for copper and was the first peptide isolated from human plasma. We can also find it in human saliva and urine. Loren Pickart first isolated the Copper Peptide GHK-Cu from human plasma in 1973 (Pickart, 1992).

Pickart noticed that liver tissue taken from people between the ages of 60 and 80 contained increased levels of fibrinogen. However, when the liver cells of these patients were incubated in the blood of young patients, the cells began to function almost exactly like "young" liver cells. In the late 1980s, the copper peptide GHK-Cu began to attract the attention of scientists as a very promising wound healing agent (Pickart, 1973, Aupaix, 1990; Simeon, 2000; Canap, 2003; Cangul, 2006; Simeon, 2009).

In its optimal, picomolar to nanomolar concentration, GHK-Cu stimulates the synthesis of collagen in human fibroblasts (Pollard, 2005; Gruchlik, 2012), increases the concentration of proteins in the skin, glycosaminoglycans and protects the DNA of cells (in the process of wound healing) (Pickart, 2008).

The scientists also noted that the GHK sequence is present in the collagen structure and suggested that the GHK peptide is released from the collagen structure in the process of injury. They proposed a whole class of protective molecules that are released from the extracellular matrix at the site of injury.

GHK-Cu also increases the synthesis of decorins – small proteoglycans involved in the regulation of the collagen synthesis process, regulation of the wound healing process and anti-cancer defense (Kang, 2009; Matalka, 2012). Copper peptide GHK-Cu is widely used in so-called natural cosmetics that have the anti-aging acting. Many controlled clinical trials confirm its anti-ageing, wrinkle-reducing and improving skin elasticity and firmness (Wegrowski, 1992; Huang, 2007).

Copper compounds with proteins called copper peptides were also found in the composition of snail slime. Its are used in anti-aging treatments, as an effective rejuvenating agent, an effective stimulator of wound healing and tissue damage. It turned out that it has not only activity in supporting regeneration. To some extent, it also affects gene expression and has anti-cancer effects (Kang, 2009; Matalka, 2012). Copper peptides stimulate collagen production and help reduce the appearance of dark marks, acne scars and UV spots (Abdulghani, 1998). Applied topically, copper peptides act as an antioxidant, stimulate collagen and elastin production, and reduce the appearance of fine lines and wrinkles (Maquart, 1988; Wegrowski, 1992; Simeon, 2000; Gul, 2008; Varvarescou, 2011; Badenhorst, 2016). Copper is an anti-inflammatory agent that accelerates wound healing, great for healing scars, pigmentation and redness caused by inflammation. If you are prone to acne, it can help with blemishes by normalizing the concentration of bacteria on the skin.

Nowadays, there are also mentions that copper peptides can affect hair growth by acting on stem cells (Perez-Menza, 1988; Uno, 1993; Choi, 2012).

BIOGENIC METHOD OBTAINING COLOIDAL PARTICLES

Snail slime can be used in obtaining natural, so-called biogenic method of colloidal silver particles (nano-silver). In cosmetology, nanosilver and nanogold are used primarily because of their biocidal properties. In addition, nanoparticles do not easily penetrate the skin barrier and are effective against various groups of microbes even at low concentrations. Research reports on the environmentally friendly synthesis of silver nanoparticles from the hitherto unexplored mucus of the land snail *Achatina fulica* using an easy,

clean and easily scalable method. Detailed characterization of the obtained samples using spectroscopy techniques confirmed the formation of silver and glod nanoparticles in the snail slime matrix (Onzo, 2021).

The obtained samples were tested against a wide range of Gram-positive and Gram-negative bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and the fungal strain *Aspergillus fumigatus* by the well diffusion method (Abd-El Azem, 2022). The results indicate that silver nanoparticles in the mucus matrix have a strong antibacterial as well as antifungal effect (Łysakowska, 2009). The antitumor effect was performed *in vitro* using cell lines cervical cancer. Biogenically synthesized Ag nanoparticles in biocompatible slime have demonstrated anticancer activity. The possibility of producing antibacterial and possibly anticancer creams/gels for topical use in skin ailments is suggested. Nanosilver is currently used in anti-acne cosmetics, hair shampoos, shower gels, antibacterial wipes, creams, intimate hygiene wipes colognes, makeup remover wipes, aftershave gels (Bugla-Błoskowska, 2007; Alanzi, 2010; Pokorowiecki, 2012; Arct, 2015; da Silva Martins, 2018; Heckel, 2019; Arroyo, 2020; Gubitoza, 2020; Li, 2020; Mane, 2021; Rizzi, 2021).

TYPES OF PRODUCTS FTOM SNAIL SLIME

The obtained raw material is used to produce cosmetic products in various forms, including: snail slime serum, eye cream, face mask, body lotion, face cream (for daily care) and cream for special skin problems (acne, scars stretch marks, cellulite, uneven skin tone). On the one hand, manufacturers praise their products promising that they will cope with: shadows under the eyes, drooping eyelids, dry skin, swelling, so-called. crow's feet, discoloration, lack of firmness, which is not always true. On the other hand, scientific research has confirmed that snail slime has the following properties: regenerating, protective, brightening skin discolorations, exfoliating, soothing, moisturizing, anti-wrinkle, anti-inflammatory, increasing skin elasticity and promoting the synthesis of collagen and elastin (Quevauviller, 1953; El Mubara, 2013; Krzyżanowska, 2019; Lim, 2020; Leśków, 2021; Khrokalo, 2022; Yongeun, 2022).

Cryptosine contains a number substances with a healing effect: 1) Elastin regenerates and repairs the dermis. 2) Glycolic and lactic acids and sodium lactate are natural agents for cleansing dead cells and increasing the mobility of other bioactive ingredients into the skin. 3) Collagen moisturizes and keeps skin supple. 4) Allantoin has healing and anti-aging properties. 5) Vitamins A, E, C have caring properties. 6) Peptides have healing and anti-inflammatory properties. 6) Folic acid cleanses skin pores and has antibacterial properties. 7) Urea softens and moisturizes the skin and is bacteriostatic. 8) Hyaluronic acid is responsible for moisturizing the skin and retaining moisture in the cells. It is a component of connective tissue, which in combination with collagen and elastin gives the skin elasticity. 9) Free amino acids stimulate skin regeneration processes (Sanchez, 2006). 10) Natural antibiotics are effective against bacteria found in skin infections: *Propionibacterium acnes, Staphylococcus aureus* (golden staphylococcus), *Eschrichia coli* (colon stick) and *Pseudomonas aeruginosa* (blue pus bacillus) (Iguchi, 1982; Pitt, 2015). Based on it drugs are used in cases of severe skin burns (Ahmad, 2018). Specialist beauty salons and beauty salons also use care treatments involving the application of live snails directly to the skin. They are indicated in the following cases: dry, gray, dull skin with the first signs of photoaging, wrinkles, loss of firmness and elasticity, discoloration, age spots, dilated capillaries, acne and rosacea, stretch marks, scars and hyperkeratosis of the epidermis (Wernecke, 2007; Uivavosan, 2011; Fabi, 2013; Dolashka, 2015; Liu, 2017; Lim, 2020).

The main effects of such treatments are: strong hydration of the skin, stimulation of collagen and elastin production, cleansing and brightening of the face, regeneration and smoothing of fine wrinkles, lightening of pigmentation spots, alleviation of inflammation and irritation, and reduction of stretch marks and scars. These treatments are used in conjunction with other methods, e.g. mesotherapy (Brieva, 2008; Fabi, 2013; Liu, 2017; Trapella, 2018; Gentili, 2020; Alogna, 2021, Yongeun, 2022).

CONCLUSIONS

The effect of cosmetics and drugs with snail slime is therefore diverse and multidirectional. It can be successfully used as a natural raw material in the pharmaceutical industry – an alternative to substances obtained by chemical synthesis. Its acquisition is relatively cheap and has a humane and ecological character. The strong regenerative properties of the snail mucus, especially mucins and cryptosine, will be able to be successfully used in the future on a larger scale to produce preparations with high care, regenerative and therapeutic values, and at the same time devoid of allergenic properties.

The use of the potential hidden in the snail slime is a reference to the practices of well-known ethno-pharmacies (Meyer-Rochow, 2017), e.g. 1) in the removal of warts, 2) in the treatment of skin problems (pruritus, acne), 3) in the treatment of injuries, bone fractures (rheumatism, sciatica, edema), 4) in the case of toothache, 5) in respiratory ailments (asthma and tuberculosis), 6) in gastric problems – stomach ache, reflux), 7) in wound healing, 8) in strengthening vitality, 9) in building the body's resistance to infections and also may constitute-make the imitation of nature (Stawarczyk, 2012). Perhaps the topic discussed will constitute another milestone in man's eternal pursuit of the desired achievement of longevity.

References

Abdulghani A.A., Sherr S., Shirin S., Solodkina G., Tapia E.M., Gottlieb A.B. **Effects of topical creams containing vitamin C, a copper-binding peptide cream and melatonin compared with tretinoin on the ultrastructure of normal skin – A pilot clinical, histologic, and ultrastructural study**. Disease Management and Clinical Outcomes. 1998; 1: 136-141.

Abd-El Azem H.H., El-Seedi H.R, Fallatah A.M., Khalifa S.A.M., Gharib M.M. **Antifungal activity of soft tissue extract from the garden snail *Helix aspersa* (Gastropoda, Mollusca)**. Molecules. 2022; 27: 370.

Adikwu M.U., Nnamani P.O. **Some physiological and toxicological properties of snail mucin**. Bioresearch. 2005; 5: 1-6.

Agu M.O., Tsware B.J., SundayA.O., Onwuka J.C., Anthony H.J. **Investigatory study of long term doses of *Costus afer*, snail slime, and their combination with a standard pharmaceutical drug on blood glucose level of alloxan induced Swiss Albino rat**. Open Journal of Medicinal Chemistry. 2018; 8: 1-14.

Ahmad T.B., Liu L., Kotiw M., Benkendorff K. **Review of anti-inflammatory, immune-modulatory and wound healing properties of molluscs**. Journal of Ethnopharmacology. 2018; 210: 156-178.

Alanazi F.K., Radwan A.A., Alsarra I.A. **Biopharmaceutical applications of nanogold**. Saudi Pharmaceutical Journal. 2010; 18: 179-193.

Alogna A., Gentili W., Trapelli K., Hallana S.S., Sguizzato M., Stazzabosco G., et al. **Design of liposomes carrying *Helix complex* snail mucus: preliminary studies**. Molecules. 2021; 26: 4709.

Amah A.K., Ewa O., Karimah M.R., Elendu M.U., Yunusa Z. **Effect of *Archachatina marginata* mucin on the aggressive factors of gastric ulcer challenged Wistar rat stomach tissue**. GSC Biological and Pharmaceutical Science. 2019; 9: 077-082.

Antonova O., Dolashka P., Toncheva D., Rommensee H-G., Floetenmeyer M., Stevanovicia S. **In vitro antiproliferative efect of *Helix aspersa* hemocyanin on multiple malignant cell lines**. Zeitschrift für Naturforschung C. 2014; 69: 325-334.

Arcadi J.A. **The two types of mucous gland cells in the integument of the slug, *Lehmania poirieri* (Mabille): A study in metachromasy**. Transactions of the American Microscopical Society. 1967; 86: 506-509.

Arct J., Pytkowska K., Pawłowska M., Stolarek M. **Nanosilver in medicine and cosmetology- use and safety**. Dermatologia Estetyczna. 2015; 17: 230-236 (in Polish).

A revolutionary way of obtaining snail slime. Available at: https://polishsnailholding.com/ [Accessed: 02.12.2022] (in Polish)

Arroyo G.V., Madrid A.T., Gavilanes A.F., Naranjo B., Debut A., Arias M.T., et al. **Green synthesis of silver nanoparticles for application in cosmetics**. Journal of Environmental Sciental Health A Toxic/ Hazardous Substances and Environmental Engenering. 2020; 55: 1304-1320.

Aupaix F., Maquart F.X., Salagnac V., Pickart L., Gillery P., Borel J.P., et al. **Effects of the tripeptide glycyl-histidyl-lysine-Cu++ on healing. Clinical and biochemical correlations**. Journal of Investigative Dermatology: 1990; 94: 390.

Available at: https://aspersasnails.com [Accessed: 02.12.2022] (in Polish)

Available at: https://helixfarm.pl [Accessed: 02.12.2022] (in Polish)

Available at: https://ecosnailsfarm.pl [Accessed: 02.12.2022] (in Polish)

Available at: https://hodujslimaki.pl [Accessed: 07.12.2022] (in Polish)

Available at: https://slimakiwieczorek.pl [Accessed: 02.12.2022] (in Polish)

Beckera P.T., Lambert A., Lejeune A., Lanterbecq D., Flammanga P. **Identification, characterization, and expression levels of putative adhesive proteins from the tube-dwelling *Polychaete Sabellaria alveolata***. Biological Bulletin. 2012; 223: 217-225.

Badenhorst T., Svirskis D., Merrilees M., Bolke L., Wu Z. **Effects of GHK-Cu on MMP and TIMP expression, collagen and elastin production, and facial wrinkle parameters**. Journal of Aging Sciences. 2016; 4: 166.

Błaszak Cz. **Zoologia. Bezkręgowce**. Warszawa 2009, p. 435-437. ISBN 9788301173357 (in Polish)

Boyanova O., Dolashka P., Toncheva D., Rammensee H.G., Stevanović S. **In vitro efect of molluscan hemocyanins on CAL-29 and T-24 bladder cancer cell lines**. Biomedical Reports. 2013; 1: 235-238.

Braun M., Menges M., Opoku F., Smith A.M. **The relative contribution of calcium, zinc and oxidation-based cross-links to the stiffness of *Arion subfuscus* glue**. Journal of Experimental Biology. 2013; 216: 1475-1483.

Brieva A., Philips N., Tejedor R., Pivel G.J.P., Alonso-Lebrero J.L., Gonzaleza S. **Molecular basis for the regenerative properties of a secretion of the mollusk *Cryptomphalus aspersa***. Skin Pharmacology and Physiology. 2008; 21: 15-22.

Bugla-Płoskońska G., Oleszkiewicz A. **Biological activity of silver and its medical application**. Kosmos – problemy nauk biologicznych. 2007; 56: 115-122 (in Polish).

Canapp S.O., Farese J.P., Schultz G.S., Gowda S., Ishak A.M., Swaim S.F., et al. **The effect of topical tripeptide-copper complex on healing of ischemic open wounds**. Veterinry Surgery. 2003; 32: 515-523.

Cangul I.T., Gul N.Y., Topal A., Yilmaz R. **Evaluation of the effects of topical tripeptide-copper complex and zinc oxide on open-wound healing in rabbits**. Veterinary Dermatology. 2006; 17: 417-423.

Choi H.R., Kang Y.A., Ryoo S.J., Shin J.W., Na J.I., Huh Ch.H., et. al. **Stem cell recovering effect of copper-free GHK in skin.** Journal of Peptide Science. 2012; 18: 685-690.

Cilia G., Frantini F. **Antimicrobial properties of terrestrial snail and slug mucus**. Journal of Complementary and Integrative Medicine. 2018; 20170168: 1-10.

Ciszewski M. **Snail farms are becoming more and more popular**. Available at: https://agronews.com.pl/artykul/hodowle-slimakow-coraz-bardziej-popularne [Accessed: 02.12.2022] (in Polish)

Cook A., Shirbhate R. **The mucus producing glands and the distribution of the cilia of the pulmonate slug *Limax pseudoflavus***. Journal of Zoology. 1983; 201: 97-116.

Copper peptides for hair loss. Available at: www.regrowth.com. Regrowth. [Accessed: 03.02.2023]

Copper Tripepite- a small molecule with extraordinary power. Available at: https://dpderm.pl/blog/tripeptyd-miedziowy-1... [Accessed: 31.12.2023] (in Polish)

Cristiano L., Guagni M. **Zooceuticals and cosmetic ingredients derived from animals**. Cosmetics. 2022; 9: 13.

da Silva Martins L.H., Rai M., Neto J.M. **Silver: biomedical applications and adverse effects**. Biomedical Applications of Metals 2018; 4: 113-127.

Das P.P.G., Bhattacharyyi B., Bhagawati S., Sarmach K., Nath D.J. **Methods of extraction of mucin from giant African snail *Achatina fulica Bowdish*.** Indian Journal of Enthomology. 2022; 84: 296-300.

Dias D., Urban S., Roessner U. **A historical overview of natural products in drug discovery**. Metabolities. 2012; 2: 303-336.

Differences in the life of the snail *Helix aspersa Maxima* and *Helix aspersa Müller*. 2022. Available at: https://hodowla-slimakow.p/hellix-aspersa-muller [Accessed: 02.12.2022] (in Polish).

Dolashka P., Dolashki A., Velkova L., Stevanovicia S., Molin L., Tradi D., et al. **Bioactive compounds isolated from garden snails**. Journal of BioSciene and Biotechnology. 2015; 5: 147-155.

Dolashki A., Dolashka P., Stenzla A., Stevanovicia S., Aichera W.K., Velkova L. **Antitumour activity of Helix hemocyanin against bladder carcinoma permanent cell lines**. Biotechnology and Biotechnological Equipment. 2019; 33: 20-32.

Domínguez-Martín E.M., Tavares J., Ríjo P., A.M. Díaz-Lanza A.M. **Zoopharmacology: a way to discover new cancer treatments**. Biomolecules. 2020; 10: 1-20.

Dwek M.V., Ross R., Streets A.J., Brooks C., Titcomb A., Woodside J., et al. ***Helix pomatia* agglutinin lectin-binding oligosaccharides of aggressive breast cancer**. International Journal of Cancer. 2001; 95: 79-85.

Ekobon T., Tongararm P., Roytrakul S., Meesuk L., Chumnanpuen P. **Prediction of anticancer peptides against MCF-7 breast cancer cells from the peptidomes of *Achatina fulica* mucus fractions**. Computional and Structural Biotechnology Journal. 2016; 14: 49.

Ellijimi C., Ben Hammouda M., Othman H., Moslah W., Jebali J., Mabrouk H.B., et al. ***Helix aspersa Maxima* mucus exhibits antimelanogenic and antitumoral effects against melanoma cells**. Biomedicine and Pharmacotheraphy. 2018; 101: 871-880.

El Mubaraka M., Lamari F.N., Kontoyannisa Ch. **Simultaneous determination of allantoin and glycolic acid in snail mucus and cosmetic creams with high performance liquid chromatography and ultraviolet detection**. Journal of Chromatography. 2013; 1322: 49-53.

Fabi S.G., Cohen J.L., Peterson J.D., Kiripolsky M.G., Goldmana M.P. **The effects of filtrate of the secretion of the *Cryptomphalus aspersa* on photoaged skin**. Journal of Drugs in Dermatology. 2013; 12: 453-457.

Fabricant D.S., Farnsworth N.R. **The value of plants used in traditional medicine for drug discovery**. Environmental Health Perspectives 2001; 109: 69-75.

Finkley M.B., Appa Y., Bhandarkar S. **Copper peptide and skin. Cosmeceuticals and active Cosmetic, 2nd Edition**, In: Eisner P. and Maibach H.I. (Editors) **Marcel Dekker**, New York. 2005: 549-563. ISBN 9780429192258

Gabriel U., Sbanca M., Jinau I. **Quantification of mucoproteins (glycoproteins) from snail mucus, *Helix aspersa and Helix pomatia***. Journal of Agroalimentary Processes and Tehnologies. 2011; 17: 410-413.

Gesheva V., Hausheva S., Mihaylova N., Manoylov I., Duumanova L., Idakieva K., et al. **Anti-cancer properties of gastropodan hemocyanins in murine model of colon carcinoma**. BMC Immunology. 2014; 15: 34.

Gentili V., Bortolotti D., Benedusi M., Alogna A., Fantinati A., Guiotto A., et al. ***Helix complex* snail mucus as a potential technology against O3 induced skin damage**. PLoS One. 2020; 15: e0229613.

Gomot A. **Biochemical composition of Helix snails: Infuence of genetic and physiological factors**. Journal of Molluscan Studies. 1998; 64: 173-181.

Gorouhi F., Maibach H.I. **Role of topical peptides in preventing and treating aged skin**. International Journal of Cosmetic Science. 2009; 31: 327-345.

Gubitosa J., Rizzi V., Fini P., Del Sole R., Lopedota A., Laquintana V., et. al. **Multifunctional green synthetized gold nanoparticles/chitosan/ellagic acid self-assembly: Antioxidant, sun filter and tyrosinase-inhibitor properties**. Mater Science and Engineering C-Materials for Biological Applications. 2020; 106: 110170.

Gugliandolo E., Cordaro M., Fusco R., Peritore A.F., Siracusa R., Genovese T., et al. **Protective effect of snail secretion filtrate against ethanol-induced gastric ulcer in mice**. Scientific Reports. 2021; 11: 3638.

Gul N.Y., Topal A., Cangul I.T., Yanik K. **The effects of topical tripeptide copper complex and helium-neon laser on wound healing in rabbits**. Veterinary Dermatology. 2008; 19: 7-14.

Grabda E. **Zoologia. Bezkręgowce, t. 1, cz. 3**, Warszawa 1983, 621-623. ISBN (in Polish)

Greistorfer S., Klepala W., Cyrana N., Gugumuck A., Rudoll L, Suppana J., et al. **Snail mucus glandular origin and composition in *Helix pomatia***. Zoology. 2017; 122: 126-138.

Greistorfer S., Suppana J., Cyran N., Klepala W., Farkas R., Rudoll L., et al. **Characterization of the *Arion vulgaris* pedal gland system**. Journal of Morphology. 2020; 281: 1059-1071.

Greistorfer S., von Byern J., Miller I., Meyer-Rochow V.B, Farkas R., Steiner G. **A histochemical and morphological study of the mucus producing pedal gland system in *Latia neritoides* (Mollusca; Gastropoda; Hygrophila)**. Zoology. 2023; 156: 126067.

Gruchlik A., Jurzak M., Chodurek E., Dzierzewicz Z. **Effect of Gly-Gly-His, Gly-His-Lys and their copper complexes on TNF-alpha-dependent IL-6 secretion in normal human dermal fibroblasts**. Acta Poloniae Pharmaceutica. 2012; 69: 1303-1306.

Gural-Sverlova N.V., Gural R.I., **Morphological, anatomical and behavioural peculiarities of the slugs from the *Arion lusitanicus complex* in Western Ukraine**. Ruthenica. 2011; 21: 97-111.

Huang P.J., Huang Y.C., Su M.F., Yang T.Y., Huang J.R., Jiang C.P. **In vitro observations on the influence of copper peptide aids for the LED photoirradiation of fibroblast collagen synthesis**. Photomedicine and Laser Surgery. 2007; 25: 183-190.

Hecel A., Kolkowska P., Krzywoszynska K., Szebesczyk A., Rowinska-Zyrek M., Kozłowski H. **Ag+ complexes as potential therapeutic agents in medicine and pharmacy**. Current Medicinal Chemistry. 2019; 26: 624-647.

Iguchi S.M., Aikawa T., Matsumoto J.J. A**ntibacterial activity of snail mucus mucin**. Comparative Biochemistry and Physiology A Comparative Physiology. 1982; 72: 571-574.

Ikejiuba F. **Some physicochemical and wound healing properties of snail mucin**. Bolletino Chimico Farmceutico. 2005; 144: 1-8.

Jura Cz. **Bezkręgowce. Podstawy morfologii funkcjonalnej, systematyki i filogenezy**. Warszawa 2007. ISBN (in Polish)

Jura Cz. **Bezkręgowce. Zarys morfologii, systematyki i filogenezy**. Warszawa 1983. ISBN (in Polish)

Kang Y.A., Choi H.R., Na J.I., Huh C.H., Kim M.J., Youn S.W., et al. **Copper-GHK increases integrin expression and p63 positivity by keratinocytes**. Archives of Dermatological Reseach. 2009; 301: 301-306.

Khrokalo L., Chyhyrynets O., Salitra N. **Chemical properties of *Helix aspersa* mucus as a component of cosmetics and pharmaceutical products**. Materails Today: Proceedings. 2022; 62: 426.

Kohlmüzer S. **Pharmacognosy**. Warszawa 2013. ISBN 9788320046519 (in Polish)

Krzyżanowska M. **A slippery topic-snail slime under the microscope**. Available at: age-less.pl/sliski-temat-czyli-sluz-slimaka-pod-lupa/ [Accessed: 02.12.2022] (in Polish)

Lai J.H, del Alamo J.C., Rodríguez-Rodríguez J., Lasheras J.C. **The mechanics of the adhesive locomotion of terrestrial gastropods**. Journal of Experimental Biology. 2010; 213: 3920-3933.

Laack E., Nikbakht H., Peters A., Kugler Ch., Jasiewicz I., Edler L., et al. **Lectin histochemistry of resected adenocarcinoma of the lung: *Helix pomatia* agglutinin binding is an independent prognostic factor**. American Journal of Pathology. 2002; 160: 1001-1008.

Lescar J., Sanchez J.F., Audfray A., Coll J.L., Breton Ch., Mitchella E., et al. **Structural basis for recognition of breast and colon cancer epitopes Tn antigen and Forssman disaccharide by *Helix pomatia* lectin**. Glycobiology. 2007; 17: 1077-1083.

Li J., Celiz J., Yang J., Wamala I., Whyte W., Seo B.R., et al. **Tough adhesives for diverse wet surfaces**. Science. 2017; 357: 378-381.

Li X., Wang Y.Y., Huang J.H., Chun-Yuan Ch., Zhen-Xing W. **Silver nanoparticles: Synthesis, medical applications and biosafety**. Theranostics. 2020; 10: 8996-9031.

Lim V.Z., Yong A.A., Tan W.P.M., Zhao X., Vitale M., Goh C.L. **Efficacy and safety of a new cosmeceutical regimen based on the combination of snail secretion filtrate and snail egg extract to improve signs of skin aging**. The Journal of Clinical and Aesthetic Dermatology. 2020; 13: 31-36.

Liu L., Sood A., Steinweg S. **Snails and skin care- an uncovered combination**. JAMA Dermatology. 2017; 153: 650.

Mair J., Port G.R. **The influence of mucus production by the slug, *Deroceras reticulatum,* on predation by *Pterostichus madidus and Nebria brevicollis (Coleoptera: Carabidae)***. Biocontrol Science and Technology. 2002; 12: 325-335.

Mane P.C., Sayyed S.A.R., Kadam D.D., Shinde M.D., Fatehmulla A., Aldhafiri A.M., et al. **Terrestrial snailmucus mediated green synthesis of silver nanoparticles and in vitro investigations on their antimicrobial and anticancer activities**. Scientific Reports. 2021; 11: 13068.

Matusiewicz M., Kosieradzka I., Niemiec T., Grodzik M., Antuszewicz H., Strojny B., et al. **In vitro infuence of extracts from snail *Helix aspersa Müller* on the colon cancer cell Line Caco-2**. International Journal of Molecular Science. 2018; 19: 1064.

Martin A.W., Deyrup-Olsen I. **Function of the epithelial channel cells of the body wall of a terrestrial slug *Ario limax columbianus***. Journal of Experimental Biology. 1986; 121: 301-314.

Maquart F.X., Pickart L., Laurent M., Gillery P., Monboisse J.C., Borel J.P. **Stimulation of collagen synthesis in fibroblast cultures by the tripeptide-copper complex glycyl-L-histidyl-L-lysine-Cu2+**. FEBS Letters. 1988; 238: 343-346.

McDermott M., Cerullo A.R., Parziale J., Achrak E., Sultana Sh. J., Ferd J., et al. **Advancing discovery of snail mucins function and application**. Frontiers in bioengineering and Biotechnology. 2021; 9: 51-57.

McShane B., Bath J., Jaramillo A.M., Ridley C., Walsh A.A., Evans Ch.M., et al. **Mucus**. Current Biology. 2021; 31: 938-945.

Menta C., Parisi V. **Metal concentrations in *Helix pomatia, Helix aspersa and Arion rufus:* a comparative studies**. Environmental Pollution. 2001; 115: 205-208.

Meyer-Rochow V.B. **Therapeutic arthropods and other, largely terrestrial, folk-medicinally important invertebrates. A comparative survey and review**. Journal of Ethnobiology and Ethnomedicine. 2017; 13: 9.

Morozińska-Gogol J. **Parazytologia medyczna. Kompendium**. Warszawa 2016. 46. ISBN 978-83-200-5138-4. (in Polish)

Newar J., Ghatak A. **Studies on the adhesive property of snail adhesive mucus**. Langmuir. 2015; 31: 12155-12160.

Min-Jee O., Sung-Min P., Hee-Taek K. **The effects of snail secretion filtrate on damaged skin barrier's recovery of the atopic dermatitis**. The Journal of Korean Oriental Medical Ophthalmology & Otolaryngology & Dermatology. 2010; 23: 138-153.

Müller K. **Pharmaceutically relevant metabolites from lichens**. Applied Microbiology and Biotechnology 2001; 56: 9.

Mu A., Okolie C.O., Agboke A. **The effect of snail mucin on the ulcer healing rate of clarithromycin**. Journal of Pharmacy Research. 2008; 8: 6.

Nanosilver in cosmetics. Available at: https://biotechnologia.pl/kosmetologia/artykuly/ [Accessed: 03.02.2023]

Newton M.I. **Wet adhesion and adhesive locomotion of snails on anti-adhesive non-wetting surfaces**. Public Library of Science One. 2012; 7: 369831-369835.

Niewiadomska K., Pojmańska T., Machnicka B., Grabda-Kozubska B. **Zarys parazytologii ogólnej**. Warszawa 1981, 69. ISBN (in Polish).

Leśków A., Tarnowska M., Szczuka I., Diakowska D. **The efect of biologically active compounds in the mucus of slugs *Limax maximus* and *Arion rufus* on human skin cells**. Scientifc Reports. 2021; 11: 18660.

Ligaszewski M., Łysak A., Surówka K. **Chemical composition of meat from *Helix pomatia* L. from the natural population and the breeding population derived from it**. Roczniki Naukowe Zootechniki. 2005; 32: 33-45. (in Polish).

Łysakowska M., Denys P. **Antimicrobial application of silver**. Kwartalnik Ortopedyczny. 2009; 4 :408-417. (in Polish)

Matalka L.E., Ford A., Unlap M.T. **The Tripeptide, GHK, induces programmed cell death in SH-SY5Y Neuroblastoma cells**. Journal of Biotechnology and Biomaterials. 2012; 2: 144.

Moore J. **Wprowadzenie do zoologii bezkręgowców**, trans. Borowik M.M., Gołębiewska-Skoczylas M., Niedźwiecki G., Warszawa 2006. s. 135-149. ISBN (in Polish)

Noothuan N., Apitanyasai K., Panha S., Tassanakajon A. **Snail mucus from the mantle and foot of two land snails*, Lissachatina fulica and Hemiplecta distincta,* exhibits different protein profile and biological activity**. BMC Research Notes. 2021; 14: 138.

Obtaining the snail slime. Available at: https://zycierolnika.pl/index.php/zwierzeta/item/ [Accessed: 02.12.2022] (in Polish)

Onzo A., Pascale R., Acquavia M.A., Cosma P., Gubitosa J., Gaeta C., et al.

Patent application. CN 104586732A. Method for extracting snail slime extract containing active enzymes. Nantong Yufengdi Biotechnology Co.,Ltd.

Paszkiewicz W., Ziomek M., Szkucik K., Maćkowiak-Dryka M. **Production and quality of snail meat**. Medycyna Weterynaryjna. 2014; 70: 673-679. (in Polish).

Pawlicki J.M., Pease L.B., Pierce C.M., Startz T.P., Zhang Y., Smith A.M. **The effect of molluscan glue proteins on gel mechanics**. Journal of Experimental Biology. 2004, 207: 1127-1135.

Perez-Meza D., Leavitt M., Trachy R. **Clinical evaluation of Graft Cyte moist dressings on hair graft viability and quality of healing**. Interantional Journal of Cosmetic Surgery. 1988; 6: 80-84.

Pickart L. **Compositions for accelerating wound healing in mammals containing cupric salt or complexes with amino acid or peptide**. US Patent 5,164, 367, 1992.

Pickart L. **The human tri-peptide GHK and tissue remodeling**. Journal of Biomaterial Science Polymer Edition. 2008; 19: 969-988.

Pickart L. **Thaler Tripeptide in human serum which prolongs survival of normal liver cells and stimulates growth in neoplastic liver**. Nature New Biology. 1973; 243: 85-87.

Pitt S.J., Graham M.A., Dedi C.G., Taylor-Harris P., Gunn A. **Antimicrobial properties of mucus from the brown garden snail *Helix aspersa***. British Journal of Biomedical Science. 2015; 72: 174-181.

Pokorowiecki R., Mielczarek A. **Selected aspects of silver nano-particles application in medical procedures**. Nowa Stomatologia. 2012; 3: 117-121. (in Polish).

Pollard J.D., Quan S., Kang T., Koch R.J. **Effects of copper tripeptide on the growth and expression of growth factors by normal and irradiated fibroblasts**. Archives of Facial Plastic Surgery. 2005; 7: 27-31.

Pons F., Koenig M., Michelot R., Mayer M., Frossard N. **The bronchorelaxant effect of helicidine, a *Helix pomatia* extract, interferes with prostaglandin E2**. Pathologie Biologie. 1999; 47: 73-80.

Pons F., Koenig M., Michelot R., Mayer M., Frossard N. **The brocho-relaxant effect of Helicidine, a *Helix pomatia* extract, involves prostaglandin E2 release**. Pharmaceutical Biology. 1998; 36: 13-19.

Quevauviller A., Garcet S. **The mucus of *Helix pomatia* L.: preparation, composition, therapeutic and pharmacodynamic properties, biologic assay**. Review Pathology, Genetics and Physiology. 1953; 53: 653-658.

Ricci A., Marialucia G., Feghali N., Sampò S., Cataldi A., Zara S. **Snail slime extracted by a cruelty free method preserves viability and controls inflammation occurrence: a focus on fibroblasts**. Molecules. 2023; 28: 1222.

Rizzi V., Gubitosa J., Fini P., Nuzzo S., Agostiano A., Cosma P. **Snail slime-based gold nanoparticles: An interesting potential ingredient in cosmetics as an antioxidant, sunscreen, and tyrosinase in**hibitor. Journal of Photochemistry and Photobiology. 2021; 224 :112309.

Royas C., Stephenson S.L. **Myxomycetes. Biology, systematics, biogeography and ecology, 2021**. Academic Press (19 September 2021); ISBN: 9780128242810.

Sanchez J.F., Lascar J., Chazalet W., Breton Ch., Imberty A., Michell E.P., et al. **Biochemical and structural analysis of *Helix pomatia* agglutinin**. Journal of Biological Chemistry. 2006; 281: 20171-20180.

Siméon A., Monier F., Emonard H., Gillery P., Birembaut P., Hornebeck W., et al. **Expression and activation of matrix metalloproteinases in wounds: Modulation by the tripeptide-copper complex glycyl-l-histidyl-l-lysine-Cu^{2+}**. Journal of Investigative. Dermatology. 1999; 112: 957-964.

Siméon A., Wegrowski Y., Bontemps Y., Maquart F.X. **Expression of glycosaminoglycans and small proteoglycans in wounds: Modulation by the tripeptide-copper complex glycyl-l-histidyl-l-lysine-Cu($^{2+}$)**. Journal of Investigative Dermatology. 2000; 115: 962-968.

Siméon A., Emonard H., Hornebeck W., Maquart F.X. **The tripeptide-copper complex glycyl-l-histidyl-l-lysine-Cu^{2+} stimulates matrix metalloproteinase-2 expression by fibroblast cultures**. Life Sciences. 2000; 22: 57-65.

Siméon A., Emonard H., Hornebeck W. **The tripeptide-copper complex glycyl-L-histidyl-L-lysine-Cu2+ stimulates matrix metalloproteinase-2 expression by fibroblast cultures**. Life Science. 2000; 22: 2257-2265.

Skalmowski G. **Chów i hodowla ślimaka jadalnego z rodzaju *Helix aspersa***. Brwinów 2020, s. 7.

Skalmowski G. **Cosmetics- snail slime. Snail Garden**. Available at: https://www.snailsgarden.com [Accessed: 02.12.2022] (in Polish)

Smith A.M., Quick T.J., StPeter R.J. **Differences in the composition of adhesive and non-adhesive mucus from the limpet *Lottia limatula***. Biological Bulletin. 1999; 196: 34-44.

Snail slime. Innovative acquisition method. Available at: www.aspersa.com/ [Accessed: 02.12.2022] (in Polish)

Snail snail show… what you have in you. www.zdrowiebezlekow.pl/blog/slimak-slimak-pokaz-co-masz-w-sobie-n405 [Accessed: 02.12.2022] (in Polish)

Snail slime-richness ingredients for the human skin. Available at: www.przegladkosmetyczny.pl/t/kryptozyna/ [Accessed: 02.12.2022] (in Polish)

Snail slime: properties, use in cosmetics. Available at: https://www.drogeriaolmed.pl/blog/artykul/sluz-slimaka-wlasciwosci-zastosowanie-w-kosmetykach,1605006601.html [Accessed: 02.12.2022] (in Polish)

Snail slime- the ideal component. Available at: https://skinboost.pl/blog/sluzslimaka/ [Accessed: 02.12.2022] (in Polish)

Small, big-snail slime extract in cosmetics. Available at: sklep.lemone.pl/blog/wpis/may-wielki-limak-wycig-ze-luzu-limaka-w-kosmetykach [Accessed: 02.12.2022] (in Polish)

Stawarczyk K., Stawarczyk M., Piechowicz B. **Shellless snails in folk medicine: Literature review**. Etnobiologia Polska. 2012, 2: 33-38. (in Polish)

Stephenson S.L., Stempen H. **Myxomycetes: A handbook of slime molds. 1994**; Timber Press (1 September 1994); ISBN-10: 0881922773.

Swapna P., Rivender Reddy R. **Quantifcation of mucoproteins (glycoproteins) from slugs mucus, *Arion hortensis***. International Journal of Pharmaceutical and Sciences and Research. 2015; 4: 242-320.

Szkucik K., Ziomek M., Maćkowiak-Dryka M., Paszkiewicz W. **Edible snails - their performance, nutritive value and safety for consumers**. Życie Weterynaryjne. 2011; 86: 631-635. (in Polish).

Szubert K. Application of nanosilver. Available at: www.biotechnologia.pl/Artykuły http://www.biotechnologia.pl/Artykuły/Nanosrebro/Nanosrebro [Accessed: 29.01.2023]

Trapella C., Rizzo R., Galo S., Algona A., Bartolotii D., Casciano F., et al. ***Helix complex* snail mucus exhibits pro-survival, proliferative and pro-migration effects on mammalian fibroblasts**. Scientific Reports. 2018; 8: 1-10.

Uivaroşan I.G., Sbanca M., Jianu I. **Quantification of mucoproteins (glycoproteins) from snails mucus, *Helix aspersa* and *Helix pomatia***. Journal of Agroalimentary Processes and Technologies. 2011; 17: 410-413.

Wegrowski Y., Maquart F.X., Borel J.P. **Stimulation of sulfated glycosaminoglycan synthesis by the tripeptide-copper complex glycyl-l-histidyl-l-lysine-Cu^{2+}**. Life Sciences. 1992; 51: 1049-1056.

Werneke S.W., Swann C., Franquarson L.A., Hamilton K.S., Smith A.M. **The role of metals in mollusca adhesive gels**. Journal of Experimental Biology. 2007; 210: 137-145.

Wondrak G. **Monotypic gland-cell regions on the body surface of two species of Arion: ultrastructure and lectin-binding properties**. Journal of Molluscan Studies. 2012; 78; 364-376.

Uno H., Kurata S. **Chemical agents and peptides affect hair growth**. Journal of Investigative Dermatology. 1993; 101(1 suppl): 143-147.

von Byern J., Cyran N., Klepal W., Rudoll L., Suppan J., Greistorfer S. **The structure of the cutaneous pedal glands in the banded snail *Cepaea hortensis* (Müller, 1774)**. Journal of Morphology. 2018; 279: 187-198.

Varvaresou A., Papageorgiou S., Protopapa E., Katsarou A. **Testing the effectiveness and tolerability of an oligopeptide with potential anti-aging effects**. Journal of Cosmetics, Dermatological Sciences and Applications. 2011; 1: 133-140. (in Polish)

Yamaguchi K., Seo N., Furuta E. **Histochemical and ultrastructural analyses of the epithelial cells of the body surface skin from the terrestrial slug, *Incilaria fruhstorferi***. Zoological Science. 2000; 17: 1137-1146.

Yongeun K., Woo-Jin S., Jeong-Seok L., Tae-Gyu L. **Snail mucin is a functional food ingredient for skin**. Journal of Functional Foods. 2022; 92: 105053.

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