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- Holiday on prescription
- The Nijmegen Biomedical study
- Trained immunity

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FROM THE EDITORIAL BOARD

Dear reader,

It is my pleasure to present the 25th edition of RAMS. As you might be aware, our beloved Radboud University is celebrating its 100th year! Therefore, this is the main theme of this special 25th edition of RAMS. To make this edition special we decided to publish articles that highlight some of the important discoveries and research done at Radboud. We are very proud of our editors who worked hard and wrote some wonderful pieces for our readers.

Have you ever heard about trained immunity? If so, then you might also be aware that the work in the field of trained immunity was pioneered at Radboud University Medical Center. If you have not heard about it, I am sure Robbin's article will help you easily understand the concept of trained immunity.
For our special edition, Robbin also got a chance to interview Dr Wiljan Hendriks, Department of Cell Biology. Dr Hendriks has been part of the university for more than four decades and has seen it grow immensely. He highlights some important events in his career and the changes that he has witnessed in the scientific world.
The article written by Kim about the Nijmegen Biomedical Study is very interesting to read. It highlights its historical perspective as well as how meticulously the study was executed and the fact that it is still used by researchers worldwide explains its importance.
Immunotherapy has emerged as a very important addition to the arsenal against cancer, which researchers at the Radboud are also working tirelessly against. A very informative article about it is written by Rosanne who submitted the student article for this edition.
We all experience some or the other type of stress in our lives, especially as students. Yfke's Myth or Science article discusses it and how it may play a role in Parkinson's disease.
Richard has summarised the high-impact article about research done at Radboud. I am sure you will be fascinated after reading about the more recent research that is undertaken by the scientists at Radboud.
Lastly, Emma, the Scientific-editor-in-chief and I have tried our hand at writing for this edition. In her column, Emma discusses the history of Radboud, important events and research associated with it. My article is about a finding from the Radboud University Medical Center that could help diagnose prostate cancer better. I hope you will like these too!

Thank you and enjoy the summer break!

On behalf of the ninth board of RAMS,

Vikrant Pandya
Chair- Editorial Board

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TRAINED IMMUNITY:
A DOUBLE-EDGED SWORD IN HEALTH AND DISEASE

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Abstract

Trained immunity describes the long-term functional reprogramming of innate immune cells, leading to differences in the responsiveness of these cells against stimuli of different nature. These changes are characterised by modifications in the epigenetic and metabolic landscape of the cells. The term 'trained immunity' was first coined in 2011 by researchers at the Department of Internal Medicine at the Radboud University Medical Center. Since then, this one term has grown to encompass an ever-expanding line of research that spans different fields across the whole immunological spectrum. While the past decade has mostly focussed on elucidating the mechanisms behind trained immunity, the future of this field lies in our ability to find new ways of therapeutically targeting or harnessing trained immunity in different contexts. These contexts include vaccination strategies, immunotherapies, infectious diseases, and cardiovascular interventions, and promise to include many more in the next decade.

Introduction

The immune system is composed of various complex barriers, factors, and cell types, that work in concert to protect the organism against invading pathogens. Traditionally, the immune system of vertebrates is classified into two arms: the innate immune system and the adaptive immune system. The innate immune system represents the first line defence against pathogens, which must overcome this barrier to establish an infection. Innate immune cells detect 'self' and 'non-self' entities, after which they can attack anything deemed as 'non-self' [1]. In contrast, adaptive immunity is composed of cells that are highly specific against a certain antigen. Upon recognition of this antigen as 'non-self', these adaptive immune cells mount an attack and generate immunological memory against it [1]. The memory response enables the rapid and effective clonal expansion of cells during a subsequent encounter [1]. For a long time, the prevailing view was that only the adaptive immune system is capable of a memory response, whereas the innate immune system is just capable of rudimentary clearance of antigens.

However, a growing body of evidence over the past half a century opposes this view. This line of inquiry originated from Mackaness's seminal work in 1964, where he documented non-specific cell-mediated resistance to various pathogens [2]. He further described how this resistance was dependent on "an altered state of macrophages", which have a temporary heightened response after interferon-gamma release by lymphocytes [2]. Although this mechanism was still a by-product of the adaptive immune system, it provided early evidence for a heightened innate immune response following secondary infections. Subsequent research in plants and invertebrates, which lack an adaptive immune system, further corroborated the hypothesis of a memory-like response in the innate immune system. For instance, experiments performed in 1961 by Frank Ross revealed that infection by the tobacco mosaic virus also provides broad-spectrum protection that lasts for 20 days. This process is described as 'systemic acquired resistance', and is considered to be equivalent to immunological memory in vertebrates [3].

Lastly, epidemiological data demonstrated that live-attenuated vaccines reduce mortality that is not related to their primary target [4]. Vaccination trials in children further validated the existence of an innate memory-like response in humans. The full range of epidemiological data on this is beyond the scope of this article, but an extensive review by Aaby and Benn was published in 2019 [5]. The most extensively employed vaccine in the world is Bacillus Calmette-Guerin (BCG) vaccine, a live attenuated vaccine derived from Mycobacterium bovis. In addition to its protection against tuberculosis, multiple randomised trials have exhibited that the use of BCG reduces all-cause neonatal mortality, primarily through the prevention of various infections [6-8]. This rapid reduction in perinatal mortality could not be attributed to adaptive immunity, as the onset of the protection is too swift [9].

In 2011, Mihai Netea, Jessica Quintin, and Jos van der Meer at the Department of Medicine at the Radboud university medical center (Radboud University Medical Center) used the aforementioned research to postulate a new hypothesis, namely that of 'trained immunity'. In their paper titled "Trained Immunity: A Memory for Innate Host Defense", they define trained immunity as "a heightened response to a secondary infection that can be exerted both toward the same microorganism and a different one" [10]. A year later, they substantiated this hypothesis by showing that mice lacking B and T cells were protected against a Candida albicans reinfection in a monocyte-dependent manner [11]. Now, more than a decade later this hypothesis has grown into an ever-evolving research field which aims to further elucidate the concept of trained immunity. This review describes some of the accomplishments of the Radboud University Medical Center within the field of trained immunity, including the identification of the mechanisms behind this phenomenon. Furthermore, different fields which are impacted by trained immunity will be discussed. And lastly, the prospects and challenges of this new and exciting field will be covered.

Mechanisms of trained immunity

Conventional immunological memory arises from an interplay of different mechanisms. After antigen recognition, the production of lymphocyte clones leads to the formation of a large population of lymphocytes targeted against one antigen. Then, a subgroup of this population survives in the form of memory lymphocytes, which

upon restimulation quickly undergo clonal expansion for a stronger and quicker secondary immune response [1]. This memory can, depending on the antigen, result in life-long protection against a specific antigen. On the other hand, the lifetime of innate immune memory is still unclear, with timeframes ranging from 3 months to several years [12-14]. Although it is highly unlikely that trained immunity lasts as long as adaptive immunological memory, it is of note to mention that the lifetime of trained immunity does not match that of typical mononuclear cells [15]. Therefore, trained immunity must rely on other mechanisms than classical immunological memory to be effective.

Alternatively, two primary mechanisms control trained immunity, namely epigenetic reprogramming and metabolic alterations. In brief, epigenetics involves the regulation of gene function without modifying DNA sequence. This is typically achieved through changes in DNA methylation and histone modifications. These processes alter the accessibility of DNA to transcriptional machinery and thereby adjust the expression of the underlying genes [16]. In the case of trained immunity, epigenetic changes result in alteration in the inflammatory phenotype of innate immune cells. Upon initial stimulation, innate immune cells undergo rapid activation, marked by increases in gene expression of various pro-inflammatory genes. These shifts in gene expression are facilitated by increases in specific histone modifications, such as methylation or acetylation, as well as decreases in DNA methylation [17-19]. After the danger signal or pathogen has been effectively eliminated, some of the epigenetic changes remain. As a result, the upregulation of pro-inflammatory genes is quicker and stronger in the case of a subsequent (unrelated) stimulus [20].

One of the main ways by which epigenetics induces trained immunity is by modulating the metabolism of innate immune cells. Trained monocytes exhibit a shift from mostly oxidative phosphorylation, which is utilised in an inactive state, to more glycolysis [21]. This shift is evidenced by increases in glucose consumption, lactate production and the ratio between nicotinamide adenine dinucleotide (NAD(+)) and its reduced form (NADH). The mammalian target of rapamycin and its pathway can activate this metabolic reprogramming [22]. Furthermore, metabolic pathways can regulate epigenetics, by providing substrates or regulators for epigenetic enzymes [23]. As previously stated, the lifetime of trained immunity is generally longer than that of peripheral innate immune cells. This discrepancy is resolved by the concept of 'centrally trained immunity', where

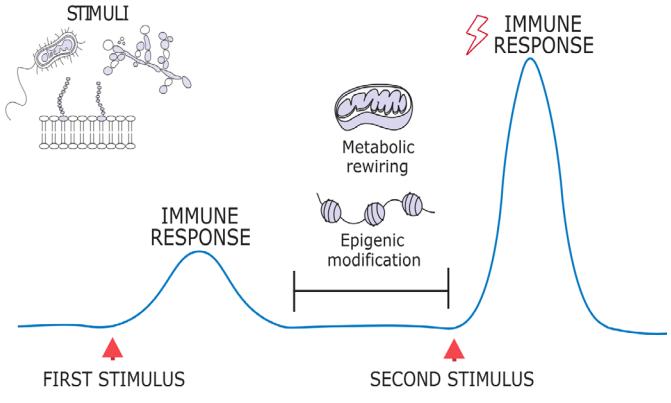

Figure 1 *- The mechanisms behind trained immunity. After a primary stimulus, epigenetic changes and metabolic alterations induce an altered state of activatability in innate immune cells. Because of this, these cells can bring about a stronger response after a second (unrelated) stimulus.*

training agents alter hematopoietic stem cells, which are the basis of the immune system. For example, the administration of β-glucan in an experiment done by Mitroulis et al. at the Radboud University Medical Center showed a pronounced increase in myelopoiesis, which coincided with alterations in the functional and transcriptional profile of bone marrow-derived progenitors [24]. This expansion ultimately provided protection against secondary lipopolysaccharide stimulation and chemotherapy-induced myelosuppression [24]. BCG, like other training agents, has also been shown to affect hematopoietic stem cells in a way that protected mice against a tuberculosis infection [25].

Applications of trained immunity

Since the initial introduction of trained immunity by Netea et al., researchers have utilised this newfound knowledge in different ways. Within the Radboud University Medical Center, different departments have picked up this knowledge and started applying it in their fields of interest. For example, the implications of trained immunity for vaccinations are immense. Therefore, the Department of Internal Medicine has continued its research into trained immunity in infectious diseases. Among others, they have further investigated the effects of BCG vaccination on protection against various non-specific infections. Here, promising, but ambiguous effects were reported.

For example, they found that vaccination with BCG protects against yellow fever viraemia and Influenza A, but not against severe acute respiratory syndrome coronavirus-2 [26-28]. Furthermore, in Denmark and Australia BCG vaccination of neonates did not significantly influence the number of hospitalisations until 15 months after birth or the occurrence of lower respiratory tract infections, respectively [29, 30]. On the other hand, BCG vaccination of elderly patients discharged from the Radboud University Medical Center did protect against new infections, most likely specifically against respiratory tract infections of probable viral origin [31]. Concurrently, BCG-vaccinated individuals showed more control over a controlled malaria infection performed at the Radboud University Medical Center [32]. More specifically, they demonstrated earlier signs of NK cell and monocytic activation, which was inversely correlated with parasitaemia. Because of this heterogeneity in response, an advisory board of the WHO has deemed the evidence to start channelling trained immunity in vaccinations inconclusive [33]. Accordingly, they have decided against altering vaccination strategies until more elaborate randomised trials are performed.

On the opposing of the spectrum, researchers have endeavoured to exploit trained immunity in immunotherapy against cancer. BCG immunotherapy has already been established as a routine treatment and prophylaxis of superficial bladder cancer [34]. At the Radboud University Medical Center, this treatment has also displayed induction of trained immunity, which reduced the likelihood of respiratory infections and conceivably had an effect on oncological outcomes [35]. Another intriguing prospect is the reversal of immunosuppressive phenotypes of tumour-associated immune cells. An instance of this phenomenon can be observed with tumour-associated macrophages (TAMs), whose presence is linked to poorer survival and prognosis in various cancers [34]. The ability of several therapeutics to repolarise these macrophages towards a pro-inflammatory phenotype is already being evaluated in clinical trials [36]. One approach that may prove beneficial in the modulation of phosphoinositide 3-kinase γ, a protein that is linked to both trained immunity and immunosuppressive phenotypes [27, 37].

A paper from 2020 resulting from a collaboration with several departments of the Radboud University Medical Center showed that a mouse melanoma model treated with a trained immunity-promoting

therapy experienced dose- and regimen-dependent tumour growth inhibition without changes in body weight [38]. This effect was induced by increased myelopoiesis due to epigenetic rewiring, which was thereby able to overcome the immunosuppression from the tumour microenvironment. Even more excitingly, the researchers propose combining their trained immunity therapeutic with other immunotherapies. This is attributed to the observation that the combination of their nano therapy with checkpoint inhibitors significantly inhibited tumour growth rate compared to the efficacy of each treatment administered in isolation [38].

Contribution to cardiovascular pathology

While trained immunity has shown some incredibly promising applications, there are also definite drawbacks to the presence of immunological memory. For example, trained immunity has been proposed to play a role in the development of cardiovascular disease, most notably in atherosclerosis. In the early stages of atherosclerosis, excessive lipids that have accumulated in vascular walls are taken up by macrophages, which become foam cells as a result [39]. This is a key step in the formation and deterioration of atherosclerosis, and it is therefore the subject of much research. In 2014, Siroon Bekkering, with the help of Jessica Quintin, Mihai Netea and others, was able to show that trained immunity plays a role in this process. They described how exposing monocytes to oxidised low-density lipoprotein instigates a proatherogenic phenotype in the resulting macrophages. Additionally, this phenotype was characterised by epigenetic histone modifications, increased proinflammatory cytokine production and foam cell formation [40].

This pivotal finding was then translated to in vivo research two years later, when the researchers saw the same pattern emerge in monocytes derived from symptomatic atherosclerosis patients [41]. Lastly, the bone marrow progenitors of patients with coronary artery disease due to severe atherosclerosis showed the tell-tale epigenetic and metabolic signs of trained immunity [42]. This has many implications for the treatment of people at risk of atherosclerosis and cardiovascular disease. Most notably because the standard treatment for this, statins, was unable to revert the trained phenotype [43].

Future directions and challenges

Although trained immunity is a widely observed phenomenon, its efficacy may be influenced by a variety of factors that require further investigation. Age, diet and exercise, among others, need to be thoroughly studied to determine their impact on trained immunity. This will become particularly important as trained immunity-based therapies are developed. Additionally, trained immunity is a complex system that involves various cell types, organs and pathologies. While it was beyond the scope of this review, much research is also conducted on the role of trained immunity in autoimmune diseases [21, 44, 45]. Notably, trained immunity is a systemic mechanism that involves reprogramming the immune system's core, the bone marrow. Therefore, research into trained immunity, at the Radboud University Medical Center and beyond, must be careful to consider potential off-target effects and safety concerns associated with these interventions. Such investigations are crucial for the safe and effective implementation of trained immunity-based therapies.

Conclusion

Trained immunity is definitely one of the astonishing discoveries that have come out of the Radboud in the last century. This field of research has shattered the long-existing belief that the adaptive immune system was alone in its ability for memory. While the beginning phases were focused on elucidating the mechanisms behind trained immunity, the research has now pivoted to look at ways to both utilise and suppress trained immunity in different contexts. Training agents like BCG could be promising in vaccination strategies and immunotherapies. On the other hand, innate immune memory can have a deleterious effect in a cardiovascular setting, so therapies targeting trained immunity should be considered here. All of this has led to many new research lines, both within and beyond Nijmegen, and there will surely be many more innovations in the future.

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CURRENT CAR THERAPIES USING T AND NK CELLS AND COMPARING THEIR ADVANTAGES AND LIMITATIONS

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Abstract

Recent developments in immunotherapy against cancer have been very promising. One such therapy is the integration of chimeric antigen receptor (CAR) constructs in T or NK cells. These CAR T and NK cells are engineered to be highly tumour-targeted and are therefore very promising compared to other non-specific therapies. Although proven to be very efficient, CAR T cells have been shown to have a high risk of inducing severe side effects. CAR NK cell therapy seems to be a safer option, as they have a considerably lower risk of causing these side effects. Due to a high risk of developing Graft Versus Host Disease (GVHD), CAR T cells are primarily obtained from the blood of the patient itself (autologous product). In contrast, because of their substantial lower risk of GVHD, NK cells can be derived from a number of different allogeneic sources and with that the possibility of creating 'off-the-shelf' CAR NK cell therapies arises. Moreover, CAR NK cells are able to keep the cytotoxic activity naturally occurring in NK cells and can therefore function in both a CAR-dependent and -independent manner, potentially leading to higher efficiency. Altogether, next to the use of potent autologous CAR T cells in certain tumour types, allogeneic 'off-the-shelf' CAR NK cells might become more desirable with less side effects.

Introduction

Since it became clear that the immune system plays a vital role in the fight against cancer [1], therapies inducing or enhancing the immune response against the tumour cells have been developed. In the last decade, breakthroughs have led to the development of new and the improvement of existing immunotherapies. These therapies include strategies involving antibodies, cytokines, gene therapy, checkpoint inhibitors, and cellular immunotherapy [2,3].

Cellular immunotherapy, based on the administration of immune cells to the patient [4], can be a specific and effective way of treatment [5]. Active therapy, such as dendritic cell (DC) vaccines, stimulates antitumour activity of the immune system. For dendritic cell vaccinations, the DCs are loaded with tumour-derived antigens and subsequently injected into the lymph nodes of the patient. Here, the DCs present the antigens to the T cells and activate an immune response against the tumour. Other injection routes may include intradermal (in the skin) and intravenous (in the veins). In passive therapy, immune cells that have an antitumour effectivity themselves, such as adoptive transfer of T or NK cells, are used. This is also known as adoptive cellular therapy [4,6]. In this therapy immune cells are expanded and modified in vitro , giving them antitumour activity, and transferred into the patient [6]. T cells are most frequently used for this type of therapy, although NK cells are now also upcoming [7]. There are three types of adoptive cell therapies, including tumour-infiltrating lymphocytes (TILs), genetically engineered T-cell receptors (TCRs), and chimeric antigen receptor (CAR) T and NK cells [5]. TILs are (non-genetically) lymphocytes, derived from modified tumour tissue, that are able to infiltrate the tumour and exert their antitumour activity from within [5,8]. CAR and TCRs are based on genetically engineered T and NK cells, that obtain specificity against tumour antigens and are therefore highly effective [9,10].

In this review, these CAR therapies, possible with both T cells and NK cells, will be discussed and compared.

CAR T cell therapy

Chimeric antigen receptors (CARs) give the immune effector cells their tumour-specificity [11,12]. The cells used in this type of therapy are typically autologous T cells, which are cells from the patient itself. CAR receptors are chimeric because both the antigen-binding and the T cell-activating functions are combined into one single receptor [12]. The antigen-binding domain, a single-chain variable fragment (scFv), is derived from an antibody that is known to target a tumour associated antigen [13] and thus varies between therapies for different patients. The scFVs are linked, via a transmembrane region, to an intracellular signalling domain of the CD3ζ chain, that exerts the activating functions. For second-generation CAR T cells, these receptors also include costimulatory molecules such as CD28 or 4-1BB [14,15] (Figure 1). The incorporation of the costimulatory CD28 domain has been shown to give the CAR T cell enhanced antitumour activity, as compared to the first generation CAR T cells [16].

Usually, the T cells are taken from the patient self, modified, and transferred back into the patient [12,15]. Besides killing cancerous cells, the CAR T cells are also able to promote immune surveillance, preventing the tumour from reoccurring [11]. Another major advantage of using CAR T cell therapies, as opposed to other immune therapies, is the fact that CAR T cells can operate independently of major histocompatibility complex (MHC) recognition [15,17]. This way, the therapy is not affected by possible immune evasion exerted by the tumour cells, through downregulation of human leukocyte antigen (HLA) class I molecules or a defective processing capability of antigens [17].

In the best-studied CAR T cell therapy, the T cells recognise the CD19 antigen. CD19 is mainly expressed on the surface of B lymphocytes and is essential for the intracellular signalling of the B cells [11,18]. The fact that the CD19 antigen is expressed on most B cell malignancies, but not on hematopoietic stem cells or other tissues, makes these antigens excellent targets against lymphomas and leukaemias. These include chronic lymphocytic leukaemia and acute lymphoblastic leukaemia (ALL), with a low risk of side effects [10,18]. However, since

CD19 is not required for survival, the tumour cells are able to escape recognition by the T cells by means of CD19 loss or downregulation [18].

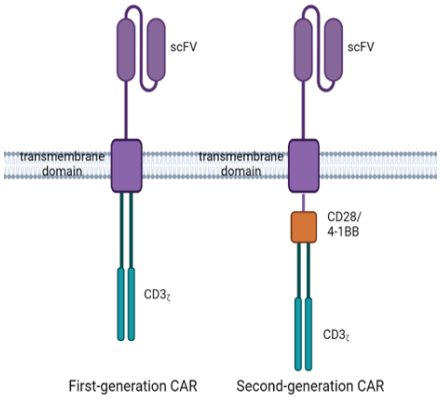

Figure 1: *Structure of first- and second-generation of chimeric antigen receptor constructs. The first-generation includes a scFV that is connected via a transmembrane domain to an intracellular signalling CD3ζ chain. For the second-generation CAR, there is an extra incorporation of a CD28 or 4-1BB domain in between the transmembrane domain and the CD3ζ chain.*

Universal T-cell therapies

As mentioned before, the T cells for this therapy are generally taken from the patient itself, modified, and infused back [12,17]. Although this makes the T cells patient-specific and therefore very efficient with a low chance of rejection, the production of these cells is extremely time-consuming and a highly skilled process. Moreover, cancer patients might develop immunodeficiency or lymphocytopenia after receiving chemotherapy, resulting in insufficient numbers of T cells. To solve these problems, universal 'off-the-shelf' CAR T cells have recently been developed [19,20]. These universal T cells are produced by genetically editing allogeneic T cells from a healthy donor in such a way that they can be used for multiple other patients [20]. However, the HLA expressed on allogeneic T cells might be recognised as foreign, leading to immune reactions against these T cells and thus rejection or even graft-versus-host disease (GVHD) [21].

Side effects

Unfortunately, CAR T cell therapies have been known to cause some severe side effects. These may include cytokine release syndrome (CRS), graft-versus-host disease (GVHD), tumour lysis syndrome (TLS), and immune effector cell-associated neurotoxicity syndrome (ICANS) [12].

CRS is a systemic inflammatory response caused by an increase in the secretion of cytokines, such as IL-1 and IL-6 [22]. The highly proliferative CAR T cells secrete more granulocyte-macrophage colony-stimulating factor (GM-CSF), which has been found to cause oversecretion of cytokines by monocytes and macrophages. By knocking out the GM-CSF production using CRISPR-Cas gene editing, the CRS risk was reduced without affecting the antitumour activity [23]. As a treatment, tocilizumab, which is an IL-6 receptor antagonist, has been shown in clinical trials to effectively work against CRS, without affecting the CAR T cell efficiency [24,25]. In 2017, the FDA approved the use of this drug for the treatment of severe CRS induced by CAR T cell therapy [26].

GVHD is the result of an immune reaction against foreign cells. This may occur in two ways after the infusion of allogeneic T cells. The existing immune system of the recipient may form a response against the infused CAR T cells, or the allogenic CAR T cells may form an immune response against the antigens expressed on the recipient's tissue cells. With the use of second-generation CAR T cells, more specifically those including the 4-1BB co-stimulatory domain, there is a higher risk for GVHD [27].

TLS has been observed after infusion of anti-CD19 CAR T cells [20]. It occurs after a large amount of cancer cells die within a short period of time, leading to the release of nucleic acids, proteins, and electrolytes, such as phosphate and potassium [26]. Thir sudden increase in blood and tissue concentration of electrolytes may cause severe toxic effects, including renal insufficiencies and cardiac arrythmias [28]. TLS generally more frequently occurs in hematopoietic cancers, although it might also occur after treatment of solid tumours [29].

ICANS is a neurotoxicity that might be life-threatening and occurs between 20 to 70% of patients treated with CAR T cell therapy [29]. Typically, ICANS correlates with CRS severity, although it may also occur in absence of CRS [24,30]. Patients with severe ICANS show an increased number of T cells and an increased level of IL-6 in their cerebrospinal fluid, due to a blood-brain permeability that leaves this fluid susceptible to cytokine infiltration [24,30,31]. It has been found that the administration of GM-CSF-neutralizing antibodies has a positive effect on reduction of ICANS in mice by decreasing cytokine release. With neutralizing this stimulatory factor, the blood-brain barrier permeability seems to be decreased to a level that is similar to controls that did not receive CAR T cell therapy [30,31].

Thus, although CAR T cell therapies seem highly efficient, there are a lot of side effects that may arise with the use of this type of therapy. Therefore, these therapies should be used with caution to prevent potential side effects as far as possible. In addition, further research is needed to minimize these side effects and optimize CAR T cell therapies.

CAR NK cell therapies

Natural killer (NK) cells belong to the innate immune system and account for 5-15% of human peripheral blood leukocytes [32]. NK cells are functionally similar to T cells and are able to kill target cells through cytotoxic mechanisms [32-34]. However, because NK cells are part of the innate immune system, they do not require activation by antigen-presenting cells (APCs) [33,35]. This gives them an advantage over T cells in research and treatment, as they will not take up as much time in production [36]. Usually, CAR T cells are activated using monoclonal anti-CD3 antibodies and anti-CD28 antibodies in vitro, mimicking stimulation by APCs, before they can be infused into the patient [35,37]. NK-cell function depends on stimulation of activating or inhibitory signals generated by germline-encoded receptors [32,36,38]. MHC class I molecules, expressed on the surface of normal healthy cells, act as an inhibiting ligand for NK cells [34], giving the NK cells their self-tolerance. Killer cell immunoglobulin-like receptors (KIR) are receptors that are able to recognise these MHC class I molecules [39]. An example of an activating receptor is the NKG2D receptor, which is one of the best-studied activating receptors. This receptor can recognise stress-induced ligands expressed on damaged, transformed, or abnormal cells. Here, ligand-receptor interaction, and the lack of MHC class I expression, will lead to NK activation, subsequent cytokine and chemokine release, and eventually lysis of the stressed cell [40,41]. This receptor-dependent activation gives the NK cells an advantage over T cells in immunotherapy. Since the NK cells are not dependent on antigen recognition, NK cells are able to respond independently of tumour-antigens, resulting in continuous antitumour effectivity even if the tumour downregulates antigen expression [36,42]. Moreover, in the rare event that tumour cells downregulate the expression of

certain MHC molecules in order to escape T cell recognition [39,43], they will become more susceptible to NK response because of reduced KIR-mediated inhibition [32,43].

Fewer side effects

CAR NK cells, although modified, still possess their natural cytotoxicity and functionality, which means that CAR NK cells are able to target tumour cells in both a CAR-dependent and -independent manner [44-46]. With this, the NK cells produce cytokines and chemokines to induce cytotoxicity against the tumour cells, while also reducing risks of relapse in patients due to CAR-dependent mechanisms [38]. This would already be a big advantage over the use of CAR T cells, however, NK cells could be even engineered to have a non-killing functionality of the CAR. Instead, they would use the CAR to promote natural NK target recognition and with that activation of its CAR-independent cytotoxicity [32]. This leads to less on-target/off-tumour toxicity, where normal healthy cells that express the same antigens are recognised by the CAR but will not induce a natural NK cell response [32,46]. Because NK cells possess a different cytokine profile, the risk of a CAR NK cell recipient developing CRS is significantly lower than that of CAR T cell recipients, or even completely absent [42,47]. This is largely because the main driving factors in the induction of CRS are the cytokines IL-1 and IL-6 [22,48], which are mostly secreted by T cells and only rarely by NK cells [34,42,48]. ICANS may be correlated to CRS severity. In many articles, no cases of neurotoxicity, including ICANS, after receiving CAR NK cells have been reported so far, which corresponds to the low risk of CRS with NK cells [48-50].

Lastly, also GVHD seemed to be less prominent with the use of CAR NK cells, despite some mismatches in HLA molecules between donors and recipients [47,51]. NK cells are even able to suppress GVHD because they inhibit alloreactive T cells, which are T cells that form a response against allogeneic MHC peptides, without causing GVHD themselves [51,52]. This reduced risk of GVHD gives rise to the opportunity for the development of 'off-the-shelf' CAR NK cells [48,50], which can be obtained from numerous sources other than autologous [53]. Using CRISPR-Cas techniques, obtaining NK cells that do not express KIRs on their surface has been shown to be possible [50]. This lack of KIRs will make the NK cells HLA genotype-independent, meaning that any donor/recipient match can be made, without causing unwanted rejection effects. This makes NK cells a better fit for 'off-the-shelf' CAR therapy than T cells, which cannot become HLA genotype-independent [50].

Sources

NK cells for CAR-mediated immunotherapy can be obtained from various sources, as illustrated in fig. 2, including cell lines (NK92 cell line), umbilical cord blood (UCB), peripheral blood (PB), and induced pluripotent stem cells (iPSCs) [54-56].

Of the existing NK cell lines, NK92 seems to be the only one that has shown strong antitumour activity [55-57]. They have been extensively used as a source for CAR NK cells because they can expand indefinitely in vitro, and do not show to be affected by multiple freezing rounds [32]. Also, the NK92 cells have little to no expression of inhibitory KIRs or CD16 on their surface [44,48]. Altogether makes these cells perfect for the production of 'off-the-shelf' CAR NK cells. However, since the NK92 cell line is derived from a cancer patient with non-Hodgkin lymphoma [54,58], there are some limitations. The NK92 cells will require irradiation before they can be infused in the patient [32,56], which potentially can be lethal to the individual cells, creating a loss of proliferation of the cells in vivo. Yet, this irradiation is necessary in order to reduce the risk of tumourigenicity [56,59].

NK cells can also be isolated from umbilical cord blood (UCB). These cells have a low expression of CD16 [48,56,59] and are relatively easy to collect as they constitute up to 30% of UCB [59,60]. Moreover, there are only a few T cells present in UCB and most are immature, resulting in a minimal risk of GVHD [60,61]. However, the quantity of cells in UCB is limited, as it contains 10- to 100-fold fewer nucleated

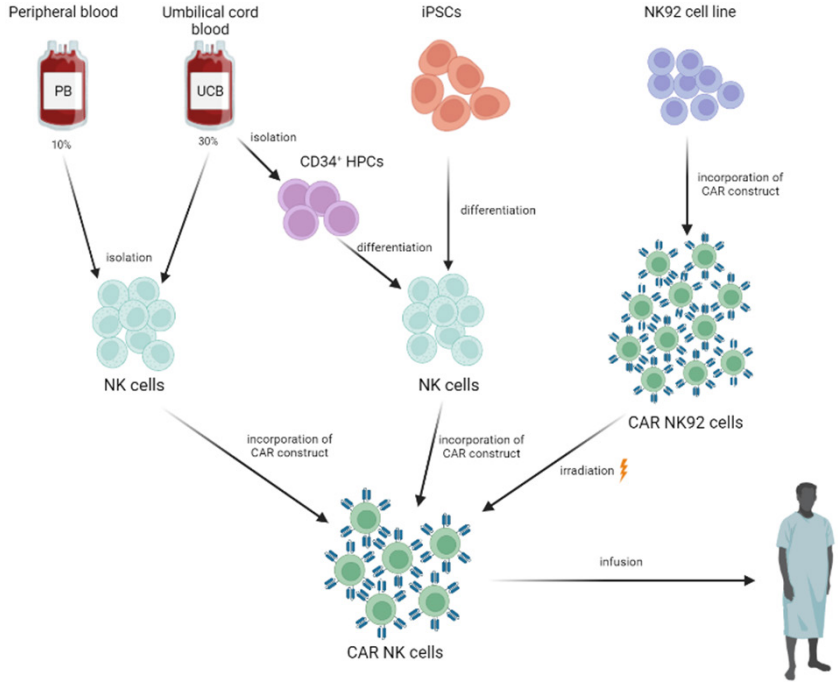

Figure 2: *NK CAR cell sources, including peripheral blood, umbilical cord blood, induced pluripotent stem cells, and NK92 cell line. Of peripheral and umbilical cord blood NK cells can be isolated after which they can be modified unto CAR NK cells. From umbilical cord blood, CD34+ hematopoietic progenitor cells can also be isolated, which can be in turn differentiated into NK cells and subsequently modified to CAR cells. Cells of the NK92 cell line can be directly modified into CAR NK cells, but they have to be irradiated before infusion, leading to a loss of cytotoxicity.*

cells compared to other sources. Moreover, the NK cells obtained from UCB often show an immature phenotype, which has an inferior cytotoxicity [32,62]. CD34+ hematopoietic progenitor cells (HPCs) can also be extracted from UCB, which can serve as an appealing and versatile option for obtaining NK cells [63,64]. These CD34+-derived NK cells have been found to possess higher antitumoural cytotoxicity than NK cells directly isolated from blood [65] and are therefore also an efficient source for CAR NK cells.

In peripheral blood (PB), the amount of NK cells is lower compared to UCM, as they constitute up to only 10%. [48]. The NK cells can be derived from autologous or allogeneic PB, making it a desirable source [66]. Although the same procedure can be applied for the isolation of NK cells from PB and UCB [67], due to the low numbers of NK cells in PB, the isolation is more difficult [56]. Besides, purification is of the highest importance when obtaining NK cells from PBMCs, since remaining B and T cells, which are present in high concentrations in peripheral blood, may cause unwanted side effects [48]. However, approximately 90% of the NK cells isolated from PBMCs have a mature phenotype, giving them a higher cytotoxic activity [32,48,67]. This makes them readily available for therapy and able to be expanded in vivo without any further stimulation [68].

NK cells derived from donor blood are not from a homologous source, making the the development of 'off-the-shelf' CAR therapies more difficult [55,66]. A way to circumvent the dependability on donors is the use of induced pluripotent stem cells (iPSCs) that can be differentiated into NK cells [55]. iPSC-CAR NK cells, expressing NKG2D, showed improved anticancer activity in leukaemia and ovarian cancer [55,69]. Additionally to the CAR construct, other modifications, such as higher or lower expression of certain receptors to further enhance antitumour activity, are easier with iPSCs than with isolated NK cells from blood [69]. Moreover, because the NK cells derived from this source are easily accessible for modification and expansion, 'off-the-shelf' production is readily achieved [70]. Although iPSCs-NK cells seem to be a better fit for CAR therapies due to their numerous advantages, the development of NK cells from this source is a lengthy and expertise-requiring process [38,71].

As of now, the NK92 cell line and peripheral blood NK cells are the most commonly used sources. NK92-derived NK cells have shown higher cytotoxicity levels compared to PB-derived NK cells [70]. However, because NK92 cells require irradiation before infusion, their cytotoxicity is significantly reduced [56,70]. Newer approaches are therefore being developed. Despite their slightly more complicated manufacturing iPSC-derived NK cells will have an advantage over NK cells derived from other sources in the future [71].

Conclusion

In the field of immunotherapy, the development of CAR therapies has been a breakthrough. Here, both CAR T and NK cells have been shownto be effective in targeting and killing tumour cells in various studies. This type of treatment can be highly specific and effective, and is therefore an ideal therapy. However, CAR T cell therapies have a high risk of causing severe side effects, including CRS, GVHD, TLS, and ICANS. These side effects may cause detrimental effects on the patient, in some cases even leading to death [28]. CAR NK cells on the other hand have been associated with a significant lower risk of developing these side effects and are therefore considered a safer option. Moreover, upon the construction of CAR NK cells, the NK cells seem to be able to keep their natural cytotoxic activity, leading to the possibility for CAR NK cells to function in a CAR-dependent and -independent manner. This may result in a higher effectivity in general and also a lower risk of on-target/off-tumour effects.

Another advantage of using NK cells instead of T cells in CAR therapies is the fact that NK cells can be derived from various different sources. T cells most often need to be derived from the peripheral blood of the patient [54] to reduce the risks of GVHD. However, since the patient may have developed immunodeficiency or lymphocytopenia after receiving chemotherapy, there might be insufficient amounts of T cells present, leading to difficulties in the development of a CAR T cell therapy. Because allogeneic CAR NK therapies have a significantly lower risk of GVHD, the sources of NK cells are endless. Currently, various sources are already being used and improved, but also new sources of NK cells are being discovered. An example of such a new source is the NK101 cell line, derived from a patient with an extra-nodal natural killer/T-cell lymphoma, as an improvement on the NK92 cell line [72]. This NK101 cell line has been shown to produce higher levels of pro-inflammatory cytokines and can positively influence leukocyte proliferation.

Also, feeder cell lines, that make expansion of the NK cells possible, are being developed [73]. Because of these various sources, is also possible with CAR NK cells to easily produce 'off-the-shelf' CAR therapies, further increasing the efficiency of the therapies.

Although most of the CAR therapies are being used in hematopoietic cancers, solid tumours may pose as targets as well. However, tumours are able to evade T cell recognition by several mechanisms within the tumour microenvironment, which may explain the often poor results of CAR T cell therapies in solid tumours. On the other hand, CAR NK cells, mainly derived from the NK92 cell line, have proven to be more successful for such solid tumours and can thus also be used to target cancers, such as pancreatic, ovarian, and prostate cancers [50,54]. However, this type of treatment is still in early clinical development and cannot yet be used on patients [74], but it holds a promising future.

Currently, another type of CAR therapy is being developed using macrophages to increase efficiency in treating solid tumours [75,76]. However, it seems that there is a hurdle in the production of CAR macrophages (CAR-Macs) because there is no possibility for expansion. For this, the CAR constructs are being incorporated into iPSCs. These CAR-containing iPSCs will be instructed to differentiate into macrophages, generating CAR-iMac lineages, which thus also contain the CAR construct [77]. Although CAR-Macs have been seen to be effective and therefore a promising therapy, they are still in pre-clinical stages [76]. It is expected that this therapy in the future will become more efficient, although some limitations are likely yet to be uncovered.

All in all, next to autologous CAR T cells, 'off-the-shelf' CAR NK cells may be the more desired therapy in certain tumour types and clinical settings, due to several advantages. These include a higher cytotoxicity of the NK cells against the tumour cells, a lower risk of developing side effects, the possibility of deriving NK cells from various sources, and the possibility of developing 'off-the-shelf' CAR NK cells. However, there are still some limitations linked to this type of therapy. New sources of NK cells are being developed, as well as new CAR therapies using macrophages. Although CAR-Macs are still in very early stages, there seems to be effectiveness and they may pose a promising future therapy.

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INTERVIEW WITH DR WILJAN HENDRIKS: COLLABORATIVE SCIENCE AT THE RADBOUD(UMC)

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Insight

This year, our beloved university becomes a centenarian. One can imagine that a lot has changed during this time, both within the university and beyond. For this special jubilee edition of RAMS, I was joined by Dr Wiljan Hendriks of the Department of Cell Biology, who has been walking around on this campus on and off since 1977. This four-time Molecular Mechanisms of Disease lecturer of the year winner and (co-)author of over 130 papers shared his insights about his time at the Radboud these last decades, how science has changed during this time, and what his most valuable tips are for scientists during the next 100 years.

Changes at the Radboud over 50 years

While I don't think any of us students were alive in 1977, some might have an idea of what the university, then still under the name Catholic University Nijmegen, looked like back then. Dr Hendriks explains that most of the buildings we think of as characteristically Radboud did not exist yet. Both the Huygens building and the Experience Centre had yet to be built, and the hospital also looked completely different. This difference in architecture also translated to differences in study programmes. Dr Hendriks was interested in recombinant DNA techniques from a young age, and to pursue that interest he could only choose between Chemistry and Biology as a programme. Now, with the addition of Medical Biology, Molecular Mechanisms of Disease, and Molecular Life Sciences, there are many more options for combining the different natural sciences.

Dr Hendriks felt an intense craving to understand how the living world around us can exist when in essence it is only built up from 'dead' molecules and atoms. At the end of Dr Hendriks' programme in Chemistry, he was finally able to take some biochemistry courses and come into contact with this field that he had been admiring since his youth. From here, he had to decide on internships to wrap up his programme, which would now encompass both a bachelor's and a master's degree. While many of us now do one internship and one thesis, tradition back then was to do one major internship related to your specialisation and two minor internships in the broader field. Now, these internships are often highly established and you stay in a group for a short and defined period of time. While it was Dr Hendriks' goal to do this as well, he ended up staying at both of his internships for two full years.

When asked whether his long-awaited second internship in recombinant DNA techniques was worth the wait Dr Hendriks gives two reasons, the first being a personal one. The other reason he enjoyed the internship so much was the techniques he was working on at the time. Most of us will know dideoxy DNA sequencing, now more well-known as Sanger sequencing, as a widely used and well-established technique. However, at the time of Dr Hendriks' work at the Biochemistry Department, this technique was not up and running yet in Nijmegen. Therefore, it was his job to get this technique going and to start using it. Dr Hendriks states that this work laid the foundation for his later academic career. "After working on this for two years, I enjoyed it so much that I realised this was the way to go".

Work and life are intertwined

When asked to elaborate on his first reason for enjoying the second internship in recombinant DNA techniques Dr Hendriks becomes bashful. He states "The moment I entered the lab and the group I would be working with I saw this fantastic technician whom I fell in love with". After she taught him all of the nitty-gritty aspects of the work, she apparently felt the same as they are still together 41 years later. This is not the only personal relationship Dr Hendriks has developed during his time at the Radboud. He explains that he feels he succeeded in science because of the tutorage and mentorship he received from some great role models over the years. The guidance of the late Professor Wilfried de Jong at the Biochemistry Department allowed him to feel comfortable enough to continue with his PhD there [1]. Additionally, in Professor Dr Bé Wieringa Dr Hendriks found another person who was fascinated with how 'dead' molecules make up living materials.

Because of this, after working on a transgenic knockout mouse in Zurich for two years, Professor Dr Wieringa invited Dr Hendriks to come back to Nijmegen and help establish the same techniques there. While reminiscing about his time working with Prof. Dr Wieringa until 2016, Dr Hendriks claims "I have been the luckiest guy alive with such a person as a boss". When asked to elaborate on this further he boasts about the brilliance, cooperation and personalness of his former boss. He explains how Prof. Dr Wieringa conversed with everyone in the department, always making time for his employees when needed. Dr Hendriks reminisces about wonderful times, having fun in the lab and hanging out with other people from the department. He also explains that this luck of having thoughtful supervisors or bosses has not changed over time. His current superintendent, Alessandra Cambi, has significantly helped him through rough periods at Cell Biology.

Dr Wiljan Hendriks

Collaboration is key

During Dr Hendriks' work on DNA sequencing and transgenic knock-out mice, his first love within science crept back into his life. His interest in understanding how cooperation between 'dead' molecules could be transformed into living materials still fascinated him. For this, he went on to do 25 years of work on protein tyrosine phosphatases. These essential signalling molecules are the counterparts of the much more studied kinases and regulate a host of physiological processes [2]. In this work, Dr Hendriks encountered many obstacles. For one, the human genome contains 125 protein tyrosine phosphatase genes, so redundancy impeded his ability to create knock-out mice quite a bit [3]. Furthermore, the sudden death of his main collaborator on this research line, Jan Schepens, struck Dr Hendriks hard. This loss has significantly impacted both his persona and professional life to this day.

However, although he is no longer performing his own research, Dr Hendriks has taken up somewhat of an advisory role in both the Department of Cell Biology and beyond. He explains: "It is so rewarding to be able to help another person. Especially with research, which contains a lot of blood, sweat and tears. You encounter many failures, and then at the end, there is this success. If you can boost the number of success experiences by having lots of collaborations, that is amazing." Dr Hendriks, for example, recalls getting a phone call from Japan exclaiming that the constructs he helped them with worked beautifully. "These things, they make your scientific heart tick", according to him.

The next 100 years of science

Dr Hendriks concludes that the current scientific climate is much more separated into islands than what he experienced at the beginning of his career. Funding from grants has become both more important and more difficult to obtain. There is fewer permanent staff available in a department to keep knowledge alive and consistent. Now, more than ever, it is essential that we as scientists collaborate and share ideas. This is even more topical now with the recent merger of the three different research institutes into the Radboud Institute for Medical Innovation. The merger will fundamentally change the research lines in Nijmegen from the 'top-down', so 'bottom-up' scientific collaboration and cooperation will become even more essential.

Hendriks's final message is one for all current students and future scientists. In 2016, the FAIR data principles, meaning Findable, Accessible, Interoperable and Reusable, were published to ensure the reusability and usefulness of data and to limit the occurrence of research waste [4]. Many students who have taken courses by Dr Hendriks will have come across his love for acronyms and abbreviations. During this interview, he proposed an additional meaning to the FAIR principles that should be the guiding principles for any prospective or current scientist: Friendly, Approachable, Interactive, and Respectful. "If you are open, honest and collaborative, that is also what you will meet in others". With these attributes in mind, future scientists within the next 100 years of Radboud University can attempt to step into the hole that Dr Wiljan Hendriks will leave when he retires at the end of this academic year.

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MYTH OR SCIENCE: HOLIDAY ON PRESCRIPTION

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Insight

After living through the COVID-19 pandemic, we are all done with pandemics for the time being. However, a new pandemic might already be presenting itself – namely Parkinson's Disease. Although it is not an infectious disease that spreads as rapidly as COVID-19, the incidence of Parkinson's disease has been increasing exponentially in the past few years. The Radboud University Medical Center has a clinical expert centre aimed at treating Parkinson's disease and is on the frontiers of research in this field. Currently, no curative treatment is available, although the disease can be treated symptomatically with medication, and in some patients, surgery. One of the emerging theories is that stress worsens the symptoms of Parkinson's, which could mean that reduction of stress could improve the treatment of Parkinson's. But is this indeed true? Should

Parkinson's disease (PD) was firstly described in 1817 by British surgeon James Parkinson. Initially it was described as paralysis agitans; later, it was dubbed PD after James Parkinson[1]. James Parkinson described six patients presenting with PD's three main symptoms: bradykinesia, a resting tremor, and rigidity[2]. Bradykinesia refers to slowing and decreasing of repetitive movements, e.g. finger tapping. This can usually be seen clearly while testing coordination and movement during a neurological examination. Nonetheless, a variety of nonmotor symptoms occur as well, including autonomic dysfunction, mood disorders, and dementia[2].

PD is now known to be caused (amongst others) by a lack of dopamine in the basal ganglia[3]. The basal ganglia consist of multiple brain regions. One of those is the substantia nigra, which produces dopamine and is therefore important in PD[3]. Treatment of PD consists of the supplementation of dopamine, but the effect of levodopa can diminish with time4. In these patients or patients with severe symptoms, deep brain stimulation can be an option. It is important to know that both pharmacological and surgical treatment improve the motor symptoms, but do not affect the non-motor symptoms, i.e. depression, autonomic dysfunction, and dementia[4,5]. Recent studies suggests that stress can lead to an exacerbation of PD symptoms, which provides a possible new corner stone to the treatment of PD. Could stress reduction strategies therefore help to improve the treatment of PD?

We have all experienced times of stress, whether it be to a smaller or bigger extend. Although most of us will not remember stressful times as particularly happy times, stress does have a function. Acute stress leads to the release of adrenaline, which stimulates the sympathetic nervous system[6]. The sympathetic nervous system regulates the 'fight or flight response'. Stimulation of the sympathic nervous system leads to e.g. dilating of the pupils, increased heart rate, dilation of the bronchia, sweating, and inhibition of the gastro-intestinal system. This is particularly useful when you are being chased by a tiger, or, perhaps more realistically for most of us, trying to catch the train or aiming to finish a paper hours before the deadline. Furthermore, stress leads to an increase in the hypothalamic-pituitary-adrenal axis, which stimulates the secretion of glucocorticoids (cortisol) in the adrenal glands7. The secretion of cortisol leads to an increase in serum glucose, amongst others. This pathway is in essence regulated tightly, meaning that the increase in cortisol should be temporarily. However, in some cases, the stress may be chronic, resulting in a chronic increase in cortisol[7].

Multiple signs indicate that stress plays a role in PD. Firstly, psychological symptoms occur in a large number of patients with PD: 30-40% of patients develop depression, compared to 19.3% in the general population in the Netherlands8,9. 25-30% of patients struggle with anxiety, while 12.4% of adults in the Netherlands experienced an anxiety disorder in the past year[10]. This suggests that patients with PD are at least more susceptible to stress. Furthermore, stress can worsen motor symptoms, e.g. the resting tremor, and the response to medication can temporarily decrease in stressful times[9]. One study also showed that cortisone levels (an inactive form of cortisol) were increased in PD-patients, which suggests that the hypothalamic-pituitary-adrenal axis is upregulated in patients with PD[11]. This indicates that PD patients experience chronic stress.

Multiple theories exist that might explain the effect of stress in patients with PD. Van der Heide et al. provide a theoretical framework with three possible pathways[9]. Firstly, elevated glucocorticoids could lower the secretion of brain derived neurotropic peptide, which could lead to hippocampal and prefrontal cortex atrophy and amygdala growth9. The amygdala is said to be our 'primitive' brain, responsible for emotional responses, whereas the prefrontal cortex is responsible for our cognitions, which allow us to take a step back and relativise[12]. You might therefore be able to imagine that it becomes more difficult to cope with stress if the amygdala takes the upper hand. This could explain the high prevalence of depression and anxiety amongst patients with PD. Secondly, increased glucocorticoid levels promote the secretion of pro-inflammatory cytokines, which could damage dopamine-producing cells in the substantia nigra, promoting the progression of the disease in this way9. Thirdly, the symptoms of PD manifest when more than fifty per cent of dopaminergic cells are lost9. This means that the loss of the first fifty per cent of dopaminergic cells must somehow be compensated within the brain, which is thought to occur in the striatal dopamine system. Stress might compromise this system, and thus lead to earlier clinical manifestation of PD.

But why is this so important? Although there is medication available that manages the motor symptoms of PD, the disease still carries a high amount of morbidity and decreases the quality of life of patients severely3. Its prevalence continues to increase rapidly - from 2.5 million people affected in 1990 to 6.1 million patients worldwide in 2016[13]. This number is even expected to increase further to 12 million patients worldwide in 2040[13]. Optimalisation of the existing

treatment with stress-interventions, e.g. mindfulness, in addition to pharmacological or surgical treatments, could therefore potentially help a large number of patients.

Stress-management strategies, such as mindfulness, could therefore be implemented more widely in the future. However, their exact clinical effect on PD patients has not been fully elucidated yet. Nonetheless, could it be harmful to discuss the role of stress factors with your patients? Regardless of its clinical effect on PD, stress will not contribute to the patient's happiness.

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THE DISCOVERY OF *PCA3* AND ITS IMPLICATIONS ON THE DIAGNOSIS OF PROSTATE CANCER

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Abstract

Prostate cancer affects millions of men globally. In 2020, around 1.4 million new cases of prostate cancer were reported. Diagnosis of prostate cancer at an early stage is important for effective treatment and an increase in the overall survival rate of the patients. The Urology Department of the Radboud University Medical Center made the important discovery that the gene Prostate cancer-associated 3 or PCA3 is highly overexpressed in prostate cancer tissues. This launched the establishment of PCA3 as a biomarker for the diagnosis of prostate cancer and the development of a urine-based test for prostate cancer.

Introduction

Around 1.4 million new cases of prostate cancer were reported in the year 2020 with 375,304 men succumbing to the disease [1]. Prostate cancer is a major cause of death in men and is only second after lung cancer in cancer-associated mortality. The majority of men diagnosed with prostate cancer are above 60 years of age [2]. Prostate cancer arises due to mutations in the epithelial cells of the prostate gland. The prostate gland is a part of the male reproductive system and secretes important constituents that are a part of the seminal fluid. The disease can be confined to the prostate gland itself or become metastatic and disseminate into the lymph nodes, other organs and even to the bones. It is important to diagnose cancer when it is organ-confined as it can be treated by watchful waiting or by surgery combined with eternal beam radiation. Advanced disease requires androgen deprivation therapy, which involves blocking of the androgen receptor signalling pathway that is essential for the survival of prostate tumour cells. But tumours can become resistant to androgen deprivation therapy and they are then classified as castration-resistant prostate cancer. If these tumours disseminate into other parts of the body then the disease is termed metastatic castration-resistant prostate cancer, which ultimately becomes lethal [3].

The current diagnostic regimen for prostate cancer diagnosis includes Digital rectal examination, blood test for prostate-specific antigen (PSA), and Magnetic resonance imaging (MRI). Digital rectal examination involves the physical examination of the gland to check its texture, stiffness, and size. Additionally, prostate-specific antigen or the PSA test is used as serum levels are generally elevated in patients with prostate cancer [4]. However, these diagnostic techniques have a substantial risk for both false positives, detecting cancer when there is none, and false negatives, not detecting cancer whilst it is present. PSA levels might be elevated in case of inflammation, other pathological conditions or any physical stimulation of the prostate. Magnetic resonance imaging or MRI which involves the imaging of the prostate gland is a sensitive technique which helps detect the abnormalities present. Abnormal results of these tests prompt conducting a biopsy of the prostate gland so the presence of a tumour can be confirmed by histopathological analysis [2]. Especially the overdiagnosis of prostate cancer is cumbersome, as in such a case men will undergo unnecessary biopsies. It is therefore important to have a very definitive and characteristic biomarker for the diagnosis of prostate cancer. Having a reliable biomarker means that the risk of over-diagnosing and hence over-treating a patient is reduced. One such biomarker is the Prostate cancer-associated gene 3 or PCA3 [2].

The Discovery of PCA3 and its use for the diagnosis of prostate cancer

The fact that the Prostate cancer-associated 3 gene or PCA3 is selectively and overexpressed by prostate tissues was discovered by Bussemakers et al. at the Urology Department of Radboud University Medical Center [5]. Researchers analysed the mRNA expression levels of normal and tumour tissue samples of the human prostate. A nowadays old-fashioned technique called differential display analysis was used in this study. Using this technique, it was found that the PCA3 gene was overexpressed 10-100 times in the tumour tissue as compared to non-malignant tissue which was also confirmed by Northern blotting analysis. PCA3 expression is restricted to the prostate gland was proved by performing reverse-transcription polymerase chain reaction (RT-PCR) analysis, which revealed the absence of PCA3 in any non-prostatic tissue sample [5]. Later in next-generation sequencing analysis, it became clear that PCA3 is the most prostate cancer-specific transcript [6]. PCA3 is a long non-coding RNA. These are transcripts that do not code for any proteins and are more than 200 nucleotides long. They have been shown to be involved in a number of processes such as transcription of tumour suppressor genes and oncogenes, or regulation of genes involved in various signalling pathways [7]. There are also studies done that show that PCA3 silencing leads to a downregulation of vimentin which is a hallmark of cancer cells [8]. The exact role played by PCA3 is yet to be ascertained.

This discovery led to the establishment of the APTIMA PCA3 test (Gen-Probe Incorporated, San Diego, CA, USA)- a urine-based test for detecting the presence and expression level of PCA3 [9]. This urine-based test is minimally invasive, as it only requires a Digital Rectal Examination before collecting urine. The rectal examination is thought to release exfoliated tumour cells and extracellular vesicles ultimately into urine [8]. It is the only long non-coding RNA-based test that is approved for the diagnosis of prostate cancer [7]. This test is used for diagnosis in conjunction with other tests and is helpful in deciding whether a biopsy is necessary for a definitive diagnosis of the disease. The advantage of using a PCA3 test is that it is minimally invasive and cheaper than other tests [10]. It can also be used to decide whether a repeat biopsy for the patient suspected

for prostate cancer is necessary or not [9]. Repeat biopsies are taken when the first biopsies do not contain tumour tissue, the latter of which may be due to sampling error.

Future Perspectives

It is necessary to characterise the PCA3 long-non-coding RNA and unearth its function in the development and progression of prostate cancer. Understanding the roles played by PCA3 will help in the discovery of molecular mechanisms behind prostate cancer progression. It can also aid in the management of disease by monitoring the disease prognosis during treatment. Unravelling the roles played by this and other long-non-coding RNAs will also be important to develop therapies targeting the interaction partners of these RNAs.

Conclusion

Prostate cancer is one of the major contributors to deaths due to cancer. It is important to study the biomarkers that are associated with the disease so that it can be diagnosed at an early stage and adequately treated. The discovery of PCA3 proved to be an important milestone in this quest for a reliable biomarker. It is evident from the establishment of the urine-based PCA3 test that it was a very important discovery.

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THE NIJMEGEN BIOMEDICAL STUDY: AN EVERGREEN COLLECTION OF GENERAL POPULATION DATA THAT REMAINS RELEVANT

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Insight

The Nijmegen Biomedical Study (NBS) is a valuable resource that continues to be used for research purposes. It has retained its significance even though it was established over two decades ago, much like evergreen trees that retain their leaves throughout the year. The NBS data is far from dead, it still lives on in the international research field despite no longer growing in size and being "old". Established relationships between a risk factor and a disease typically do not change over time, meaning that just because something is "old" does not necessarily make it outdated. The author has made every effort to thoroughly analyse and synthesise the limited available information including conducting an in-depth interview with (former) project coordinators. Nevertheless, the predominant scientific source used for this article was the NBS cohort

Introduction

The Nijmegen Biomedical Study (NBS) is a large-scale population-based cohort study conducted in the municipality of Nijmegen, the Netherlands. Its original intention was to create a general reference population (i.e., controls) for comparison with people carrying a disease of interest (i.e., cases). Cases arose from the Radboud University Medical Centre (Radboud University Medical Center) and affiliated researchers had a hard time finding control subjects from the general population, so the NBS initially intended to solve that problem. The NBS contains a large group of individuals that serves as a representative sample of the population in the Nijmegen region of the Netherlands. In addition to its original goal of creating a general reference population to be used as controls, the NBS has also proven useful for studying population characteristics and biomarkers.

The NBS is a collection of medical and lifestyle questionnaire data from 9,350 (6,468 donated a blood sample) residents aged 18-99 years of the Nijmegen municipality. NBS was founded a little over 20 years ago. Now, it is world-renowned with its data being used (as part of large meta-analyses) all over the globe. The Radboud University Medical Center is recognised for its contributions in elucidating the mechanisms underlying various diseases, including those with a hereditary or lifestyle component. One of the sources used for these contributions is the NBS, among others. There is a lot to share about this study, and through a historical review, we touch upon the scientific world before NBS was initiated, the origins of the NBS, the methodology including the follow-up phases, its success, and NBS in the present time. Note that this article may differ from most reviews, as most of the information was not publicly available and thus the author has reconstructed the information through interviews with (former) coordinators Dr ir. F. de Vegt (10/2000 – 01/2009) and dr T.E. Galesloot (09/2012 – present).

A historical view of large-scale population-based cohort studies

The NBS was not the first large-scale population-based cohort. The first and also most famous and influential example of large-scale epidemiological studies in the world is the Framingham Heart Study (FHS).2 It was initiated in 1948 in the town of Framingham, Massachusetts, USA, and involved the recruitment of over 5,000 men and women who were followed over a long period (still ongoing!) to investigate the causes of heart disease. The study was unique in that it was one of the first large-scale population studies to focus on the identification of risk factors for chronic diseases. The participants were interviewed and underwent medical examinations every two years, and data on numerous factors, including diet, smoking, exercise, and family history, was collected. The data collected from the participants was used to identify the major risk factors for heart disease, such as high blood pressure, high cholesterol, smoking, and physical inactivity. The contemporary knowledge about the risk factors for cardiovascular disease is still based on that research. The FHS has had a significant impact on public health, and "the power of the large numbers of participants became evident. By comparing the data of people who had developed cardiovascular disease with those who did not, the researchers were able to draw relatively simple but important conclusions about risk factors.", said Bart Kiemeney, professor of Cancer Epidemiology at Radboud University Medical Center.3

What the FHS and NBS have in common is that both remain lodestars for understanding the trends in risk factors and disease, with their respective impacts in the field of research. However, they are not comparable in the way that FHS is longitudinal, transgenerational, and aimed at understanding the epidemiology of coronary heart disease.4 The NBS, like the FHS, is data-rich, and well-phenotyped, but despite having multiple phases, its design is not defined as longitudinal or transgenerational. The FHS was nevertheless a source of inspiration for the origins of NBS, perhaps just not in the design of the study.

Initiation and follow-up

Because of the power of data in numbers, researchers all over the world followed Framingham's design. The Radboud University Medical Center (previously UMC St Radboud), followed too, and set up the NBS in collaboration with the municipality of Nijmegen and the local public health services (GGD).

To ensure smooth operationalisation of the NBS, a pilot study was conducted from November 2001 to February 2002. Utilising the

population registers of the Nijmegen municipality, a cohort of 650 male and female residents aged 18 years or above was selected and sent a questionnaire, which included questions on lifestyle factors and health status.

Phases

The NBS is characterized by different phases that each had their respective research objectives, mainly based on the interest and demand within the affiliated Radboud University Medical Center research groups. For example, the initial phase NBS-1 was initiated in 2002. This phase formed the basis of the collection, with a random sample drawn from the municipality registry including eligible individuals. Eligibility was based on being 18 years and above, not living in institutional care or assisted living facilities, and being able to complete a questionnaire in Dutch. In total, 22,451 individuals were invited to complete a postal questionnaire and provide a blood sample. Dutch nationality was characteristic for 96% of the invited participants. NBS-2 was initiated in 2005 and was characterized by including additional health-related questions. NBS-3 (2008) was conducted to acquire more comprehensive insights into nutritional status. A sub-phase and thus sub-study, NBS-2-NIMA, was focused on cardiovascular risk prediction using non-invasive measurements of atherosclerosis (hence the 'NIMA'). NBS-4 was also launched in 2008 to enhance the comparability and consistency of available data on risk factors between the NBS and cancer patient groups that were regularly examined as well as to gather trait data and health-related information for a more extensive range of studies. In 2012, the NBS-5 phase was carried out to gather baseline data for a research project on risk factors associated with melanoma, as well as to gather information on physical activity, pain, and dyslexia in greater detail.

Measurements

Questionnaires

The NBS-1 questionnaire covered various topics such as demographics, medical history, health status, and lifestyle. Similarly, the NBS-2 questionnaire included questions related to pregnancy, mood, behaviour, memory, and daily activities. The NBS-2-NIMA-1, -2, and -3 questionnaires contained questions on general health, family history of cardiovascular traits, medication usage, quality of life, and medical history, with a specific focus on cardiovascular traits. The NBS-3 questionnaire was specifically designed as a food frequency questionnaire, while the NBS-4 questionnaire was geared towards collecting information on lifestyle factors, health, and disease. In addition, it also included questions related to life events, mood, behaviour, and reading problems. Finally, the NBS-5 questionnaire focused on health and disease, physical activity, sun exposure, pain, and reading problems. The NBS contains questionnaire data of 9,350 participants.

Blood samples and other biomaterials

Blood samples (serum (separator), heparin, and EDTA tubes) were obtained from participants that gave informed consent. In addition, as part of the NBS-2-NIMA sub-study, urine and faeces samples, swabs from mouth, hand, foot and back, and adipose tissue biopsies were collected. The haematological and biochemical parameters collected from blood samples and biomaterials include groups such as lipids, iron status, metabolites, thyroid function, liver, inflammation status, and renal function. From all participants, 6,468 have donated

Figure 1: *Overview of NBS data in collaboration with research publications outside of Radboud University Medical Center. Including publications. Latest update from 2017.*

a blood sample.

Genomics

The blood samples also created a huge reservoir for large-scale DNA research. This turned out to be a stroke of luck as new techniques for analysing DNA emerged in the years following the collection in 2002. "In 2006, a chip came onto the market that allowed you to see a person's DNA all at once. The lucky thing was that at the NBS, we were one of the few in the world who had blood samples ready from thousands of people to isolate DNA from at that time." said prof. dr. Kiemeney.3 The NBS has genome-wide genotype data available for 5,363 samples.

The success of NBS

The NBS is something the (former) project coordinators and presumably, the full project team are very proud of. NBS has its origins in Nijmegen and provides us with a glimpse into the historical logistics of data collection, such as the manual entry of data from paper questionnaires. The success of the Nijmegen Biomedical Study (NBS) is not easily comparable to the headline-grabbing discoveries and scientific advancements that come out of research within Radboud University Medical Center. It should be noted that there are headline-grabbing studies based on observational research as well, but in such cases, the focus is usually on the findings related to a particular disease or health issue, rather than the study design (i.e., NBS) itself. Additionally, one could argue that there actually are headline papers that include the NBS. However, they usually do not focus solely on the NBS but instead include NBS data in a meta-analysis or as controls in case-control studies. In figure 1, the collaboration of NBS data in other research publications including the number of publications is shown.

The fact that the data is still being used shows the relevance and impact of the study. An example of success for NBS is that it served as a reference population in the development of reference values for, for example, thyroid function5 and hepcidin6, which has implications for the diagnosis and treatment of various conditions. The NBS was used as a control population in the Nijmegen bladder cancer study that led to the finding of a sequence variant (8q24) that conferred susceptibility to urinary bladder cancer.7 Another important finding from the Nijmegen Biomedical Study is the reference values for kidney function that are specific to age and gender. This information was obtained by studying the NBS samples, and it can help doctors better understand their patients' kidney health.8 Overall, while the NBS may not be making headlines in the same way as other research, its impact and importance should not be underestimated.

The NBS holds questionnaire data from almost 10,000 participants and genomics data for more than 5,000 participants. During its peak, the NBS played a significant role in the scientific output of Radboud University Medical Center, with approximately 240 (based on 2018 data) research publications showcasing novel findings that gained global attention. Some of these studies were featured in prestigious journals such as Nature, Science, and the New England Journal of Medicine. Many NBS studies were conducted in collaboration with databases from universities around the world, which helped eliminate errors by replicating the studies on multiple occasions. Researchers can avoid searching for new control subjects for subsequent studies due to the availability of this enormous well-phenotyped database (genetically, environmentally, and biochemically).

NBS in the present time

The last convulsions of NBS date back to 2013, when the NBS-5 data collection was completed. With this much data, one might wonder what the project team's current tasks and responsibilities are, and what is being done with the data at present time.

Currently, the coordinator's tasks include data management, assessment of applications for data requests and its subsequent data transfer contracts within Radboud University Medical Center, as well as on national and international levels. To this date, there is still some demand for the NBS data, with the genome-wide chip data (GWAS) being most frequently requested.

The NBS is a data-rich collection of population data that no longer grows but is far from dead. The data still lives on as it is still being used internationally.

The NBS collected data on genetic, biochemical, and environmental factors from thousands of Nijmegen residents over two decades ago. Although the data may be considered old by some, it is still highly valuable because established relationships between risk factors and diseases or traits don't change (much) over time. For instance, if a certain genetic variant is known to be associated with an increased risk for a specific disease or trait, that association is unlikely to change in a significant way over time. Similarly, environmental factors such as smoking or diet have well-established links to various health outcomes, and these relationships are also unlikely to change substantially over time. Therefore, the data collected by NBS two decades ago can still be used to identify and study the relationships between various risk factors and diseases/traits and can provide valuable insights into the causes and prevention of various health conditions.

With the rise of the Radboud University Medical Center Biobank, all biomaterials from the NBS are stored there and are available upon request. The questionnaire data is also still available upon request and is to this date being used in various educational activities in the faculty of medical sciences at Radboud University.

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COLUMN: 100 YEARS OF RADBOUD UNIVERSITY

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While the original name of our beloved university, Katholieke Universiteit Nijmegen (Catholic University Nijmegen) and its motto, In Dei nomine feliciter, are still remnants of the past, the university has made significant progress in its (almost) one hundred years of existence. But I want to start at the beginning: who was the Saint Radboud whose name we so often use? This man lived between the years of 850 and 917 AD, and he was Bishop of the Diocese of Utrecht [1]. He devoted a large part of his life to research, which led to him becoming a patron of Roman Catholic higher education. After all those years, Saint Radboud's name was given to a foundation that would be the origin of the Catholic University Nijmegen, which was eventually founded on 17 October 1923 [2]. In 2004, the name Catholic University Nijmegen was changed to Radboud University, a name that is, also internationally, well-known these days.

Since its founding in 1923, Radboud University has undergone a lot of changes both within and outside the university. A rather impactful event in the history of the university was its temporary closing in April 1943 [2]. At this point during the Second World War, the Rector Magnificus at that time, Bernard Hermesdorf, refused to give students a declaration of loyalty to the German occupying forces. Closing the university indefinitely was inevitable at that moment. The university was also affected by the war battles, as it lost several buildings. For example, the main building located at the Keizer Karelplein had to succumb to bombings in 1944. Moreover, several professors sadly lost their lives during the war. Both Professor Robert Regout and Professor Titus Brandsma were arrested on grounds of resistance activities, and they died in a concentration camp in Dachau. An interesting story about a very special moment of contact between Bernard Hermesdorf and a group of his students, situated in Berlin, during this time, can be found on the website of the university.

A lot has happened behind the doors of the university since 1923. In this edition of RAMS, some of the research that was conducted at Radboud University and Radboud University Medical Center has been highlighted. However, it would be impossible to give credit to all contributions to every field of science that came from researchers at our university here. Next to the research mentioned in articles in this edition, there are many more interesting researchers and departments. Quite a few researchers at our university have been awarded the Spinoza Prize [3]. The Spinoza Prize is a prestigious award, which recognises the exceptionality of the winner's research and underlines the quality of research that is being conducted at Radboud University. In the medical field, some Spinoza winners are Prof. Dr Peter Hagoort, Prof. Dr Carl Figdor and Prof. Dr Mihai Netea. These awardees span the whole breadth of the medical field, from molecule to man to population. For example, Dr Hagoort is currently a professor in cognitive neuroscience and director of both the Donders Centre for Cognitive Neuroimaging and the Max Planck Institute for Psycholinguistics [4]. His research focuses on the human language system, and he uses a variety of neuroimaging techniques to investigate language and impairments of language in e.g. aphasia, dyslexia and autism. On the other hand, Prof. Dr Figdor is a professor of experimental immunology and he is part of the Department of Tumour Immunology at the Radboud University Medical Center [5]. His research is about molecular mechanisms of antigen-presenting cells, and dendritic cell vaccination as prevention for hereditary cancers, for example [6]. Lastly, Prof. Dr Netea is the head of the division of Experimental Medicine in the Department of Internal Medicine in the Radboud University Medical Center [7]. He is an infectious disease specialist, focusing his research on antifungal immunity, immunodeficiencies in the innate immune system and trained immunity.

Another researcher I would like to highlight is Prof. Dr Tjitske Kleefstra, a principal investigator at the Donders Institute for Brain, Cognition and Behaviour and professor by special appointment in clinical genetics and psychopathology of rare syndromes [8]. She was the first to describe the 9q34 deletion syndrome, which is now known as Kleefstra Syndrome [9, 10]. This is a prime example of the ground-breaking research happening on the Radboud campus.

Looking beyond the field of (medical) science, Radboud University has many alumni that became very successful outside of academia. One could think of Frans Timmermans, politician, diplomate, and the first vice-president of the European Commission, and Gracita Arrindell, the first female president of the parliament of Sint Maarten. Dries van Agt, former Prime Minister of the Netherlands is also an alumnus of Radboud University. Another well-known alumnus is Björn Kuipers, frequently involved in FIFA and UEFA football matches as a referee (although I wonder whether his scientific education has helped him in handling motivated players and passionate supporters).

Looking back on its great history, it is clear that 100 years of Radboud University is definitely something to be celebrated. For an overview of the celebrational activities organised by the university, visit its website, which is also mentioned under 'further reading'. And, with a total of 9302 publications in 2021, and a total of 6427 diplomas earned in that same year, we can be quite sure that the university is running at full steam and that the future of its research is bright [11]!

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Further reading

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RECENT HIGH-IMPACT PAPERS FROM RADBOUD UNIVERSITY MEDICAL CENTER RE-

Richard Dela Rosa[1]

With over 3,000 publications each year, scientific research is a cornerstone of the Radboud university medical center [1]. In this section, recent high-impact papers – published by researchers from the Radboud University Medical Center – will be discussed.

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Highly Potent Antibodies Open Doors for Transmission-Blocking Vaccines

Malaria continues to be a major public health concern, with millions of cases and hundreds of thousands of deaths reported every year [1]. Despite widespread efforts to eradicate malaria over the past years, malaria prevalence and its associated deaths continue to rise, largely due to the efficiency of malaria transmission [1]. Thus, novel tools and strategies are needed to achieve global malaria elimination. One promising approach is the development of transmission-blocking vaccines (TBVs) that target the sexual stages of the malaria parasite in the mosquito gut, thus preventing transmission of the disease [1]. A recent paper by Fabra-Garcia et al. published in Immunity (impact factor = 43.47) presents a promising breakthrough towards this goal [1]. A team mostly composed of researchers from the Department of Medical Microbiology reports the discovery of highly potent monoclonal antibodies against the Plasmodium falciparum surface protein Pfs48/45, a top TBV candidate [1]. The team screened the sera of hundreds of individuals from malaria-endemic regions who had natural exposure to the parasite and purified Pfs48/45-specific polyclonal antibodies [1]. They then selected the most potent antibodies and further characterized their ability to block the transmission of cultured gametocytes in an in vitro assay [1]. The results showed that two individuals, a Dutch missionary and a young Ugandan woman, had antibodies with robust transmission-blocking abilities against the malaria parasite [1]. Using a combination of biochemical and structural techniques, the researchers were able to identify the precise binding sites of the potent antibodies on the Pfs48/45 protein and to determine how these interactions block transmission of the parasite [1]. The findings provide valuable insights into the molecular basis of antibody-mediated transmission-blocking and could aid in the rational design of more effective TBVs. Furthermore, this highlights the importance of continued investment in malaria research and development.

Could Ultrasound Alone Be Enough? A Study Challenges Traditional Breast Examination Methods

Each year, over 70,000 women in the Netherlands undergo mammograms or digital breast tomosynthesis (DBT) to investigate focal breast complaints [2]. However, these procedures often cause discomfort for women due to breast compression and do not always provide a conclusive diagnosis [2]. As a result, an ultrasound is usually performed in addition to mammography or DBT [2]. But is this second examination really necessary? In a multicenter cohort study by Appelman et al. published in Radiology (impact factor = 29.146), the order of examinations was reversed to assess whether ultrasound alone could be sufficient for initial examination [2]. The study analyzed 1,961 eligible patients from various institutions in the Netherlands [2]. All participants underwent an initial ultrasound examination, then a biopsy if needed, followed by DBT [2]. Statistical analyses included sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy calculations for ultrasound and DBT. The results indicate that ultrasound is a highly reliable diagnostic tool, able to rule out breast cancer with 99.8% certainty [2]. Four out of five women could be reassured during the ultrasound that they had a benign abnormality, such as a cyst [2]. In one in five cases, further follow-up examinations were required, and half of these women were eventually diagnosed with a specific form of breast cancer [2]. The study also evaluated the diagnostic accuracy of DBT in addition to ultrasound but found that in most cases 90%, an accurate diagnosis was obtained with ultrasound alone [2]. The authors suggest that ultrasounds could be used as a first-line diagnostic tool for women with focal breast complaints, followed by DBT or mammography if necessary [2]. These findings have important implications for reducing discomfort for women undergoing breast examinations and reducing anxiety from a multi-step testing process. Additionally, this has the potential for cost savings in healthcare systems.

Cracking the Code: Unlocking Immunotherapy's Potential for Lynch Syndrome Patients Beyond Colorectal and Endometrial Cancers

Lynch Syndrome affects around 50,000 people in the Netherlands and increases the risk of colorectal and endometrial cancers [3]. Immunotherapy has been successful in treating these specific malignancies due to unique DNA mutations [3]. But do other cancers associated with Lynch Syndrome also have these mutations that confer vulnerability to immunotherapy? A recent study by Elze et al. published in the Journal of the National Cancer Institute (impact factor = 13.506), aimed to assess the specific DNA mutations in other tumour types from patients with Lynch Syndrome and how these mutations may contribute to immunotherapy vulnerability [3]. They analyzed 1,745 Lynch syndrome patients who developed malignancies. Among them, 236 had non-colorectal and non-endometrial tumours[3]. Many of these cancers, such as stomach and ureter have been associated with Lynch Syndrome before [3]. As such, they are called Lynch-spectrum tumours. The researchers showed that specific DNA mutations were indeed present in all Lynch-spectrum tumours [3]. But surprisingly, over 40% of non-Lynch-spectrum tumours also contained specific DNA mutations [3]. These findings suggest that genetic testing and surveillance should be considered for all Lynch-spectrum and non-Lynch-spectrum tumours in patients with Lynch Syndrome to identify those who may benefit from immunotherapy. This study highlights the importance of understanding the genetics of cancer and how it can impact treatment decisions. By identifying specific DNA mutations, doctors can personalize treatment plans and improve outcomes for patients with Lynch Syndrome and other hereditary cancer syndromes.

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A Word from the General Board of RAMS

Dear reader,

Thank you for taking time to read the twenty-fifth edition of RAMS. A jubilee year! This is the last edition of the ninth board and with that also the last edition for me as a bachelor student. Although the planning was tight, we can say that we delivered an edition to be proud of. Therefore, I want to thank everyone that contributed to this edition. As chair, I am happy with the work we delivered and I hope that you have enjoyed and learned something new while reading a diverse collection of impressive articles brought to you by our editorial board and editors.

This year, Radboud University celebrates its 100th anniversary, a milestone worth remembering. Since its foundation in 1923, the university has developed into a renowned institution for scientific research and higher education. The importance of student research cannot be overemphasised. Engaging students in scientific research encourages them to think actively about the subject matter they are studying and encourages them to think critically and develop new ideas. Something that RAMS has proudly contributed to for years.

Radboud University and its medical faculty have made an important contribution to medical science and healthcare in the past century and as a student, I am proud to be a part of that. Enjoy everything the university has to offer you.

On behalf of the ninth board of RAMS,

Dione van de Sanden
Chairwoman of the ninth board of RAMS 2022-2023

General Board

RAMS is directed by the general board, which consists of four (bio)medical students. As members of the board, they frequently meet to make sure all activities run smoothly. Moreover, they are in close contact with the supervisory board and the editorial staff. If you have any questions on general, promotional, or financial subjects, please contact the general board of RAMS via voorzitter.rams@ru.nl.

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The editorial board, which consists of two (bio)medical students, is responsible for the contents of the journal, from reviewing the submitted papers to their rejection or publication. Furthermore, the editorial board is in charge of writing editorials and determining the general layout. For questions concerning the content of the journal, please contact the editorial staff via hoofdredactie.rams@ru.nl. To submit papers, consult the 'for authors'-section on our website or mail to submit.rams@ru.nl.

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Reviewers have been trained with the help of masterclasses given by professors and teachers at Radboud university medical center. With their knowledge, the reviewers are able to judge the submitted scientific and editorial articles.

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