IMMUNOWATCH

EDITION N°10 – AUGUST 2024

IMMUNO-ONCOLOGY

mabdesign
THE BIOTHERAPY EXPERT

INTRODUCTION

MabDesign's Immunowatch is a one-of-a-kind information monitoring newsletter in the field of biologics. Its aim is to provide members of our association with the most recent and pertinent data gathered or generated through the key expertise of MabDesign and its collaborators in scientific research, business intelligence, market analysis and intellectual property.

Each edition will focus on trending type of biologics. Its general format includes market study research, financial and economic data, invited contributions from scientific teams working in the industry or in academia and a section dedicated to intellectual property. The content of each edition is decided by an editorial composed of two field experts. Decision concerning the theme and conception of each newsletter is done in-house by the permanent members of our editorial team.

Finally, we would like to acknowledge the support of the Ambition Recherche & Développement (ARD) Biomédicaments 2020 Phase II programme, funded by the Centre Val de Loire region during the initial phases of launching this newsletter.

BIOPHARMACEUTICALS
Innovation Dynamics in Health
IN REGION CENTRE-VAL DE LOIRE

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Access to the World's First Cancer Immune Library for Next-Gen Immunotherapy Development !

0 **Fully human anti-cancer antibodies** in less than 2 months & 0 humanization needed !

48 From 48 **cancer patients**

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Editorial

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Cancer is a moving target, constantly adapting and evolving to escape from immune system surveillance and become resistant to standard treatments. This makes it one of the most challenging diseases to cure and the second biggest cause of death worldwide. This high adaptability leads to treatment failure in 90% of patients with solid tumors. Therefore, there is a high unmet need for new precision medicine capable of combating immunotolerance and anticipating resistance mechanisms.

Innovative autologous and personalized cancer vaccine approaches have emerged in the last few years, such as mRNA, protein, and peptide strategies. These therapies have shown promising clinical results with positive safety data in solid tumors. However, they are time-consuming and still present manufacturing challenges that may be a barrier for patient access, particularly when a patient-specific biopsy is required.

In this context, Brenus Pharma, a Lyon-based biotech company, Brenus has developed a pioneering 'off-the-shelf' discovery platform using Stimulated-Tumor-(Ghost)-Cells (STC) to produce next-generation immunotherapy based on the mechanism of action of a cancer vaccine. STCs will educate the patient's immune system to target evolving tumors and give back the ability to fight against cance, with a standardized and cost-saving mode of production.

Brenus Pharma was co-founded by J. Gardette, a serial entrepreneur in healthcare (BIOCORP sold to NovoNordisk €154M (June 2023); and B. Pinteur, PharmD, with successful track record in cell therapeutics manufacturing and inventor of the STC technology, now the Chief Scientific Officer. The company is backed by internationally recognized key scientific opinion leaders. They act as strategic advisors to ensure validation process robustness, and as main ambassadors of our technology, significantly contributing to our publications. We are also supported by leading figures in clinical immuno-oncology, with 9 early-phase centers committed to our first-in-human Phase I/II trials, planned to begin in Q4 2024 for patients with colorectal cancer. (mCRC)

Brenus relies on a strong national and international collaboration grid to leverage the STC platform and build a broad portfolio of immunotherapies for solid tumors.

Additionally, the STC platform stands out as a major technological relay for the pharmaceutical industry in immuno-oncology as pharmaceutical companies must combine their leading immuno-oncology drugs which are approaching the end of their patent protection within the next seven years. Brenus Pharma is now open to anticipate scientific and clinical collaboration models which aimed at synergizing pipeline development in immuno-oncology, and drive innovation to patients.

Acknowledgments to Mabdesign for inviting us to co-edit this issue. We are delighted to contribute and present the innovative potential of our STC technology, changing the paradigm of cancer treatment with the ambition to bring the immune system one step ahead of cancer!

EDITORIAL

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We acknowledge Mabdesign for inviting us to co-edit this issue on immuno-oncology. At the Institute of Cancerology of Montpellier (IRCM), as part of the Tours-Montpellier Laboratory of excellence MabImprove, we are focusing on the development of smart antibodies with innovative modes of action (MoA) in the field of cancer. Recently, we have explored bispecific antibody formats (bsAb) to improve the therapeutic index of cancer treatments. BsAb can be defined by their ability to bind two different epitopes, generally simultaneously and on different antigens, by distinct paratopes localized on the same antibody derived molecule. The initial challenges encountered in bsAb design and developability have now been overcome, and twelve bsAb approved in oncology, including 10 approved since 2021. Most bsAb formats are IgG-based with an asymmetric structure thanks to fine engineering of the CH and CL domains (Duobody, i.e amivantanab, or knob-into-hole, i.e. mosunetuzumab) or selected C and/or V domain swapping (CrossMAb, i.e glofitanab) that forces the association of related VH and VL specificities. Alternatively, Ig-based bsAb are produced, as natural IgG, by symmetric association of heavy and light chains, here fused with additional variable scFv or Fab at the C- or N-terminal ends to obtain bispecificity (i.e. cadolinimab). A few smaller non IgG-based scaffolds are also available for selected applications like the oldest bsAb still in use in the clinic, blinatumomab, approved in 2014.

The main MoA of bsAbs in cancer include: (1) trans-targeting bsAbs linking immune cytotoxic cells to tumor cells (also called cytotoxic T/NK cell engagers), (2) binding two tumor associated antigens on cancer cells for enhanced selectivity (i.e. the anti-METxEGFR amivantanab), and (3) binding two immune checkpoints on exhausted T/NK cells for better reactivation (i.e. the anti-PD-1xCTLA4 cadolinimab). The last two categories, which target two antigens on the same cell, are known as cis-targeting bsAbs. Like classical monoclonal Ab, Fc-based bsAb can be fine tunned with Fc modifications for alternated effector function depending on the application or for stability increase. They can be engineered to display paratopes with conditional binding depending on the physicochemical or biological context which can help reduce on-target /off-tumor effects when targeting tumor associated antigens or immune checkpoints whose expression is not restricted to tumors. For instance, the decrease in pH reported in the micro-environment of some solid tumors (TME) compared to physiological 7.4 pH has been exploited by engineering paratopes with reduced binding at physiological pH to narrow the binding in the TME. Alternatively, protease sensitive peptides hiding the paratope have been fused to the N-terminal end of one of the variable region of the antibody whereby the proteolysis of the protective peptide by TME specific proteases allows a restricted action.

Beside strict trans-bsAb and cis-bsAb, bsAb can be designed for alternate innovative MoA.At IRCM, we are in the early steps of developing bsAb that specifically deplete the TME of pro-tumor cytokines secreted locally. This is achieved by using one arm of the antibody to target an internalizing and recycling tumor associated antigen and the other arm to simultaneously capture, internalize, release -thanks to a pH-dependent binding- and further degrade the tumorigenic soluble factor in the lysosomal pathway of tumor cells. These combined properties in a single molecule avoid the drawback of the stabilization of the cytokine observed with the regular cytokine neutralizing IgG, narrow the cytokine depleting activity in the tumors, and should allow reduced dosing thanks to the permanent recycling of the bsAb.

The combined bispecificity (and sometimes multispecificity) and conditional binding characteristics of some of the new bsAbs in development for cancer applications (over 1000 in clinical stages and many more in pre-clinical stages) make them promising molecules to both increase therapeutic efficacy and reduce side effects. As such, their exploitation as antibody drug conjugates could be one of the next step in the reach of the perfect therapeutic molecule.

GLOBAL IMMUNO-ONCOLOGY MARKET

Discover the marketed products, pipeline drug candidates, major deals and biopharmaceutical companies

THE IMMUNO-ONCOLOGY MARKET

By MabDesign

By mobilising the patient's immune system to fight cancer cells, immunotherapy offers new prospects for treating different types of tumour, some of which were previously considered incurable. The immuno-oncology market is growing exponentially, fuelled by scientific and technological advances, massive investment in research and development, and a growing number of regulatory approvals for innovative treatments such as Immune Checkpoint Inhibitors (ICIs), Chimeric Antigen Receptor-T cell (CAR-T) therapies, and therapeutic cancer vaccines.

Classification of biotherapies in oncology

Biotherapies can be divided into three main families: therapeutic proteins, vaccines and advanced therapy medicinal products (ITMs). To address the market for biotherapies in oncology, we will focus on particularly innovative new therapeutic approaches: therapeutic antibodies, therapeutic vaccines, in vivo gene therapies, ex vivo gene therapies and cell therapies.

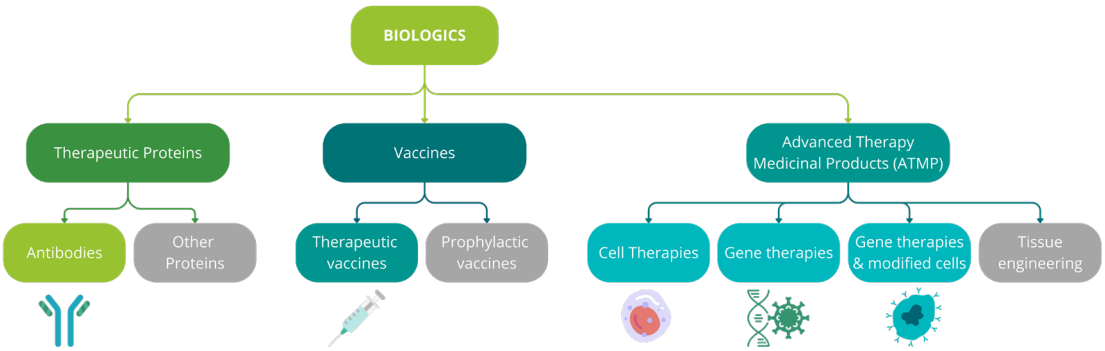

Figure 1: Classification of biologics using the scope of the immuno-oncology market analysis. *The analysis carried out in this article focuses on the market for Therapeutic Antibodies, ATMPs as Ex vivo and In vivo Gene Therapies and Cell Therapies, and finally Therapeutic Vaccines.*

Biotherapies are currently a minority on the market compared with small chemical molecules: this is also true for products positioned in oncology, with only 7% of the anti-cancer therapeutic arsenal being biomedicines. There is, however, a wide diversity of therapeutic approaches: vaccines, antibodies, recombinant proteins, cell therapies, etc.

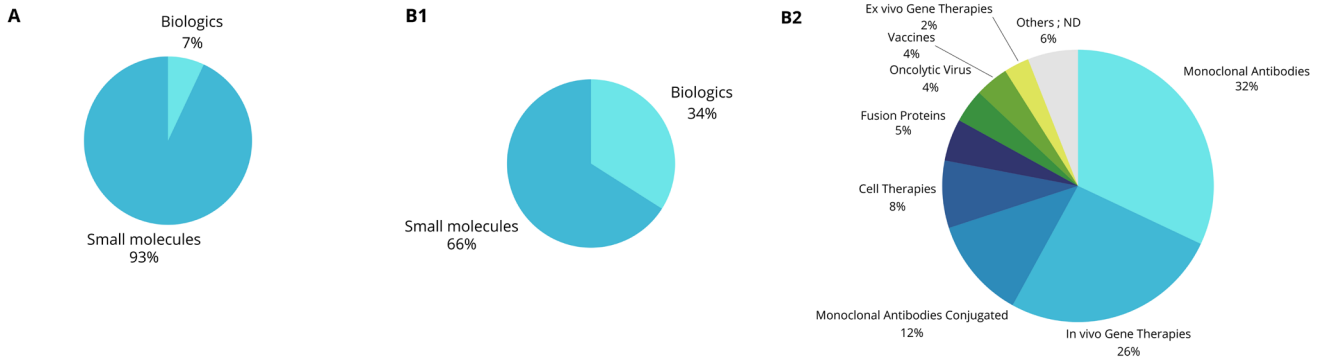

Figure 2 : Repartition of biologics and « small molecules » marketed and in development in oncology. *A) Repartition of products marketed in oncology. 502 unique products on the market are biologics, the vast majority are therapeutic proteins. B1) Repartition of products under development in oncology. B2) Focus on the distribution of biologics under development in oncology: therapeutic antibodies dominate the analysis, and ATMPs, and more specifically In vivo Gene Therapies, account for a significant proportion of the overall distribution.*

Market dynamics and trends

The immuno-oncology market has been growing for more than a decade. Its expected compound annual growth rate (CAGR) is between 9 and 12% between 2023 and 2030. This rapid expansion is being fuelled by major deals, with large pharmaceutical and biotech companies looking to strengthen their immuno-oncology portfolios, resulting in takeovers and collaborations with start-ups, biotechs and leading research institutes. The significant increase in the number of clinical trials and authorisations for new treatments also reflects the attractiveness and growth potential of the market. In 2023, 9 new biomedicines were approved in immuno-oncology.

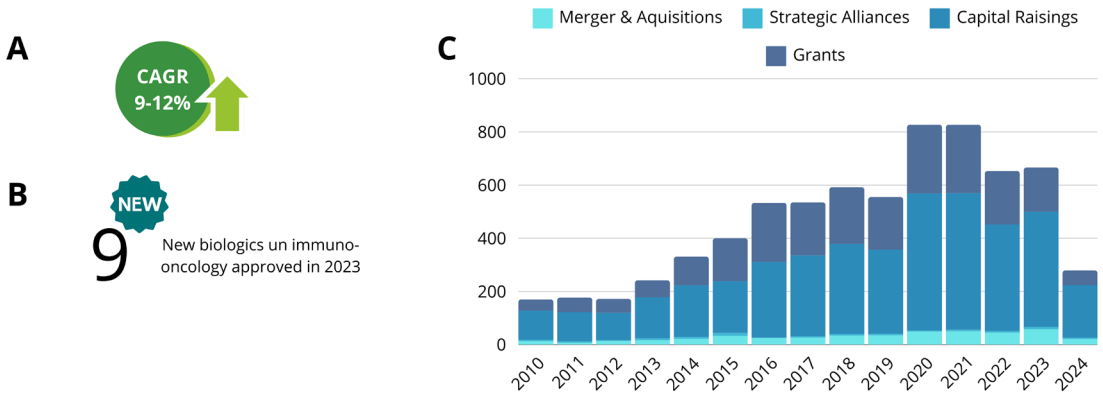

Figure 3: Dynamics of the market for biologics in immuno-oncology. *A) Compound Annual Growth Rate (CAGR) of the immuno-oncology therapeutic market expected between 2023 and 2030. B) Number of new biologics approved in immuno-oncology in 2023. C)Evolution in the number of immuno-oncology deals from 2010 to 2024.*

Focus on the therapeutic antibodies market

In addition to monoclonal antibodies, innovations such as bispecific antibodies and conjugated antibodies have emerged in recent years, broadening therapeutic options in oncology.

There are currently 108 therapeutic antibodies on the market with indications in oncology, including 14 ADCs, 11 bispecific antibodies and 30 immune checkpoint inhibitors.

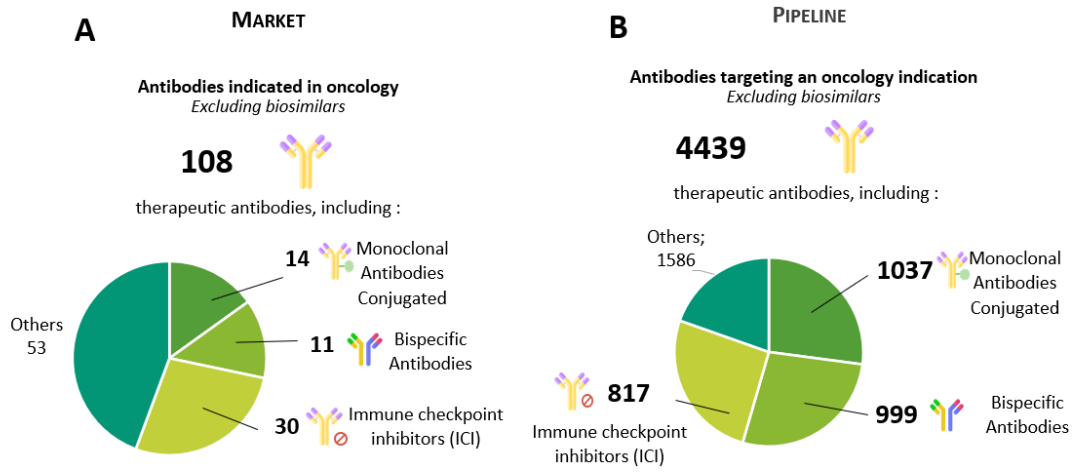

Figure 4: Therapeutic antibodies marketed and in development in oncology. *A) Repartition by type of therapeutic antibodies marketed in oncology, excluding biosimilars. B) Repartition by type of therapeutic antibodies in development, excluding biosimilars.*

The therapeutic antibody market is expanding rapidly, and is set to continue growing in the coming years, with annual growth of between 11% and 14% in the overall market. Oncology is the leading therapeutic area targeted by therapeutic antibodies, accounting for 40% of the market. This growth is expected to take the global market to a value of 500 billion dollars by 2030, with particularly significant growth forecast for the bispecific antibody segment.

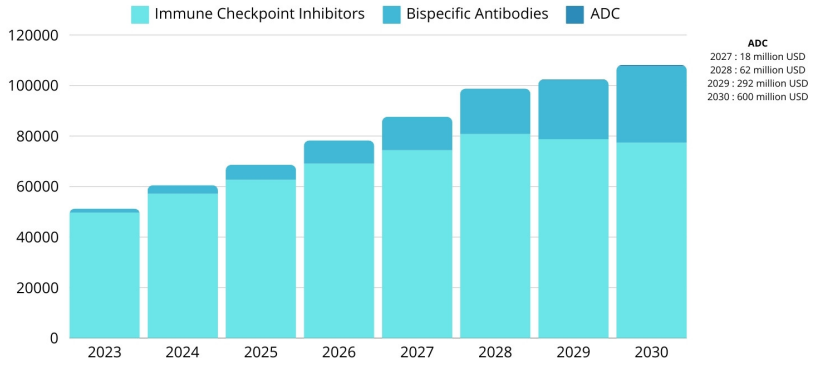

Figure 5: Sales forecasts for the three types of therapeutic antibodies analysed between 2023 and 2030, in

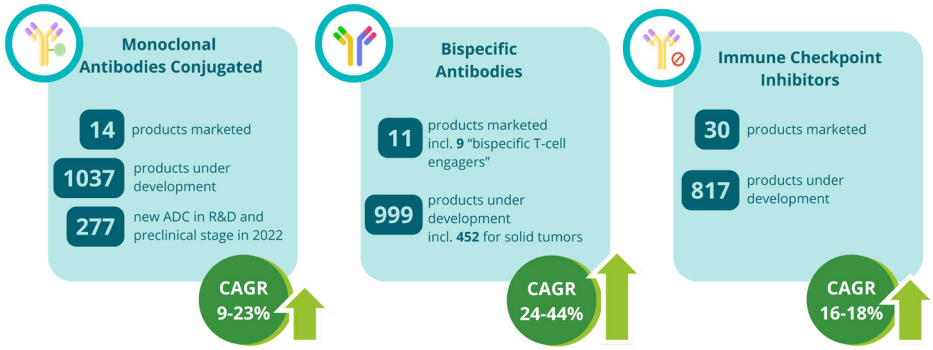

Figure 6: Key figures for therapeutic antibodies in oncology.

Focus on the MTI market

Ex-vivo gene therapies (or genetically modified cell therapies) such as CAR-T cells are still few and far between on the market: there are currently 17 products on the market. Oncology is the leading targeted therapeutic area, with 11 products, or 65% of available therapies. On the other hand, the pipeline is very rich, with more than 3,000 ex-vivo gene therapies in development, including 2,508 in oncology, i.e. 82% of the pipeline.

At present, only two in vivo gene therapies are available on the market: gendicine for the treatment of head and neck cancer (marketed in China by Shenzhen SiBiono GeneTech), and adstiladrin for the treatment of superficial bladder cancer (marketed in the USA by Ferring Pharmaceuticals).

Just as MTIs are taking a growing share of the oncology pipeline, the pipeline of in vivo gene therapies targeting this therapeutic area is substantial, with 265 products in development, including 53 in clinical phase.

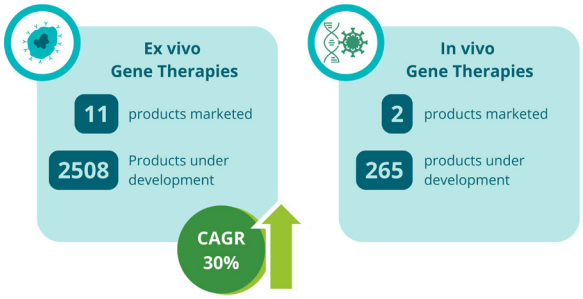

Figure 7: Key figures for gene therapy in oncology.

There are currently 7 cell therapies on the market for oncology indications. The pipeline is extensive, with more than 700 products in development. The cell therapy market is dynamic and driven by innovation, with 58 therapies entering the pipeline in 2023. In 2024, the FDA approved lifileucel (Amtagvi), the first cancer treatment to use immune cells called tumour-infiltrating lymphocytes (TILs). It is also the first cell therapy approved for a solid tumour, melanoma.

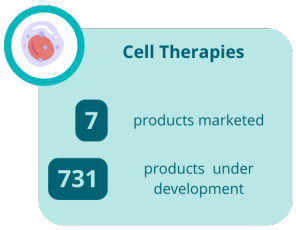

Figure 8: Key figures for cell therapies in oncology.

Focus on the Therapeutic Vaccines market

Vaccines, which have traditionally been used for prophylaxis and focused on infectious diseases, are now emerging as a promising new therapeutic tool in oncology. These new prospects are reflected in a rich pipeline of 744 projects at various stages of development, but also in a strong dynamic illustrated by the entry into the pipeline of 70 new vaccine candidates in 2023, for indications in oncology.

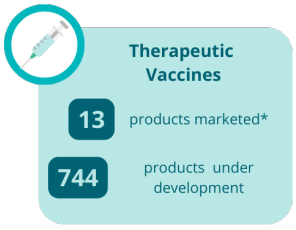

Figure 9: Key figures for therapeutic vaccines in oncology.

**It should be noted that 12 of the 13 products on the market are BCG vaccines, licensed by different companies in different countries. The remaining product is a subunit vaccine targeting GP96, available in China for the treatment of several solid cancers.*

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scientific articles

Read the different inputs from the scientific community on various aspects of Immuno-Oncology

DEVELOPING A ROBUST, TUMOUR-SPECIFIC TARGET DISCOVERY PLATFORM FOR NEXT-GENERATION IMMUNOTHERAPIES

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About Mnemo Therapeutics:

Mnemo Therapeutics is a Paris-based biotechnology company driven to create powerful immunotherapies by improving the body's ability to detect and eradicate cancer. The paucity of actionable cancer-specific targets creates significant challenges in the field of immuno-oncology. To address this, Mnemo has developed a combination of in-silico and wet-lab pipelines to uncover new tumour-specific targets (called E-antigens) derived from the unexploited dark genome, and to develop multiple therapeutic modalities (i.e. cell therapy, TCE, ADC, vaccine) targeting these E-antigens. Mnemo is currently pursuing several discovery campaigns for E-antigens, with a plan to develop its proprietary tumour-specific targets and therapeutics portfolio together with its historic founding partner Institut Curie. Mnemo is open to strategic collaborations and co-development partnerships.

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Abstract:

Development of successful immunotherapies, especially for solid tumours, is hampered by the paucity of specific and highly recurrent cancer targets. To date, cancer targets are traditionally derived from the coding genome, which encompasses roughly 4% of human DNA, leaving 96% of the genome (Dark Genome) vastly unexplored. Within that unexplored fraction lie unannotated regions and transposable elements (TEs), with poorly defined functionality. We recently discovered that due to epigenetic alterations, these transposable elements are included within coding-genome-derived proteins through non-canonical splicing generating novel proteins that we call **Epigenetic Antigens (E-antigens)**.

Here, we review the development of Mnemo's discovery engine that can readily identify highly recurrent and tumour-specific antigens by mining the Dark Genome. E-Antigens occur in two forms: peptides presented in the context of Major Histocompatibility Complex molecules (pMHC), and novel tumour-specific isoforms of transmembrane proteins (TM). Dark Genome-derived peptides are immunogenic *in vitro* and specific CD8+ T cells are identified in primary tumours and draining lymph nodes. Moreover, using *in vitro* and *in vivo* models, we demonstrate that E-Antigens are druggable and targetable by multiple immunotherapy modalities, such as chimeric antigen receptors (CARs), T-cell receptors (TCRs) and T-cell engagers (TCEs), representing an actionable target reservoir for cancer vaccines. In summary, at Mnemo,

we have developed an end-to-end discovery and validation pipeline, and we have demonstrated that E-Antigens are setting a new paradigm for antigen-based immunotherapies.

I. Why immuno-oncology needs new targets?

Target antigen selection is crucial for the development of immunotherapies that are both safe and effective for cancer patients. Therefore, an ideal target for cancer immunotherapy would need to show the following characteristics:

- Tumour specificity: the target should be expressed in the tumour cells only, not in the tumour microenvironment nor in any healthy tissues, in order to avoid on-target off-tumour toxicity.
- Tumour cell coverage: the target should be expressed by most or all the tumour cells to avoid tumour escape mechanisms.
- Patient recurrence: the target should be recurrent and shared across patients to enable product development that is impactful for patients and financially viable for companies.
- Proteogenomic and functional evidence: the target should be thoroughly characterised so that we understand its biology, assess its potential immunogenicity, develop detection assays, and readily stratify patients.
- Druggability: the target should be accessible to multiple immunotherapy modalities and its targeting needs to induce a strong antitumour effect.

The identification of such tumour-specific antigens (TSAs) poses strong challenges though, and most of the targets that are currently used in clinical practice are either not specific enough to the tumour or not shared enough across patients. Current conventional antigens in the clinic (e.g., MSLN, CD70, PSMA, CLDN6) are mainly tumour-associated antigens (TAA) showing overexpression in tumour while maintaining basal expression in healthy tissues, therefore failing to show high tumour specificity. The result is that immunotherapies against these conventional targets are prone to adverse effects due to on-target/off-tumour responses against non-malignant tissues (reviewed in *Flugel et al, 2022; Lu et al, 2024*). On the other hand, neoantigens are exclusively expressed on malignant cells and absent from all non-malignant cells. These are derived from non-synonymous mutations, insertions or deletions that alter the amino acid sequence of cell-surface proteins, from aberrant expression of oncofoetal antigens, or from tumour-specific post-translational modifications. However, neoantigens are rare, particularly in tumours with a low mutational burden, and are not shared between patients, failing thus at patient recurrence (Schumacher, Schreiber, 2015). Finally, tumour testis antigens (TTAs) are a family of antigens that is probably the closest to address all the characteristics introduced above, except that they rarely reach very high recurrence in patients.

At *Mnemo Therapeutics*, we sought to address the challenge of finding actionable, tumour-specific and tumour-recurrent targets for immunotherapy. While cancer targets currently in the clinic are derived from the coding genome which encompasses roughly 2-4% of human DNA, we have established a discovery engine that explores the entire genome, including the other 96% of the genome. This, so called 'dark genome', was historically considered as non-coding and is vastly unexplored (*Nurk et al, 2022*). Therefore, we have been able to discover a novel class of antigens: we observed that due to epigenetic alterations, dark genome-derived elements are included within coding-genome-derived proteins through non-canonical splicing and generate novel proteins that we call **Epigenetic Antigens (E-antigens)**. These E-antigens address all the characteristics of an ideal target identified above as they are tumour specific, are expressed in the majority of tumour cells, are shared across patients, are validated by multiple approaches, and are druggable by multiple immunotherapy modalities. In the next

sections, we will first introduce this new class of antigens, their origin and how they differ from current targets, we will then describe Mnemo's end-to-end discovery engine, spanning target discovery to functional validation in multiple immunotherapy modalities.

II. E-antigens represent a new reservoir of targets for immuno-oncology:

Since the first sequencing of the human genome in 2001 (*Lander et al, 2001*), scientists have approached a current consensus on an estimated number of just under 20,000 identified genes encoded by only 2-4% of the human genome. The rest of the genome is composed of 40-50% of transposable elements (i.e. LTRs, LINEs, SINEs elements), and the last 50% of the genome that represent non-transposable elements remaining poorly annotated (*Cordaux et al., 2009; Nurk et al, 2022*).

RNA splicing is the mechanism by which non-coding regions are removed from pre-mRNAs leading to the connection of exons together. It is a regulated process, happening in the nucleus, by a complex enzymatic machinery called the spliceosome (*Zhang et al., 2021*). This process produces mature mRNA transcripts that then translocate to the cytoplasm to be processed by the translation machinery, giving rise to functional proteins. Canonical RNA splicing refers to the mechanism generating all canonical and annotated isoforms, where the spliceosome recognises canonical consensus splice sites sequences. The number of canonical isoforms is currently estimated roughly between 30,000-100,000 (*Fields et al, 1994; Clamp et al, 2007; Zhang et al, 2021*). Alternative splicing on the other hand, involves at least one low affinity splice motif and generates a pool of 'alternative transcripts' that results from different combinations of exons (e.g., exon skipping or mutually exclusive exons) or from splicing events involving 'non-coding sequences' (i.e. intron retention) (*Sakabe et al, 2007; Sibley et al, 2016*). The alternative splicing increases this number to roughly 200,000-300,000 annotated isoforms. These isoforms harbour multiple roles in cellular functions, ranging from signalling, cell cycle progression to chromatin organisation, as well as in immune responses and genetic diseases (*Wong et al, 2013; Braunschweig et al, 2014; and reviewed in Alternative Splicing and Disease, 2016. Genetics – Research & issues*). More importantly, alternative splicing is particularly deregulated in cancer (*Zhang et al., 2021*) revealing an unexploited reservoir of tumour-specific epitopes (*Kahles et al., 2018, Bigot et al., 2022*)

Recent improvements both in experimental and computational techniques for transcriptomics, proteomics and immunopeptidomics have expanded our understanding of RNA processing revealing many previously unknown and unannotated splicing events (*Dobin et al 2013, Wu et al, 2013; Sibley et al, 2016*). This non-canonical splicing is mediated by the same spliceosome machinery as canonical and alternative splicing and generates a new and large reservoir of non-annotated isoforms. These isoforms are the product of splicing events involving non-coding sequences presenting alternative or cryptic splice sites (*Burns et al., 2017 and Schmitz et al., 2011*). This new reservoir is represented as the hidden part of the iceberg in **Figure 1**, and estimated to increase the pool of transcripts to roughly 500,000-2,000,000 non-canonical and unannotated isoforms. Amongst the non-coding genome, the major annotated feature family is transposable elements (TEs). They are generally intronic or located in the vicinity of genes and present cryptic splice motifs, therefore introducing non-canonical splicing events (*Lev-Maor et al., 2008, Cordaux et al., 2009, de Koning AP et al., 2011, Alvarez et al., 2021*).

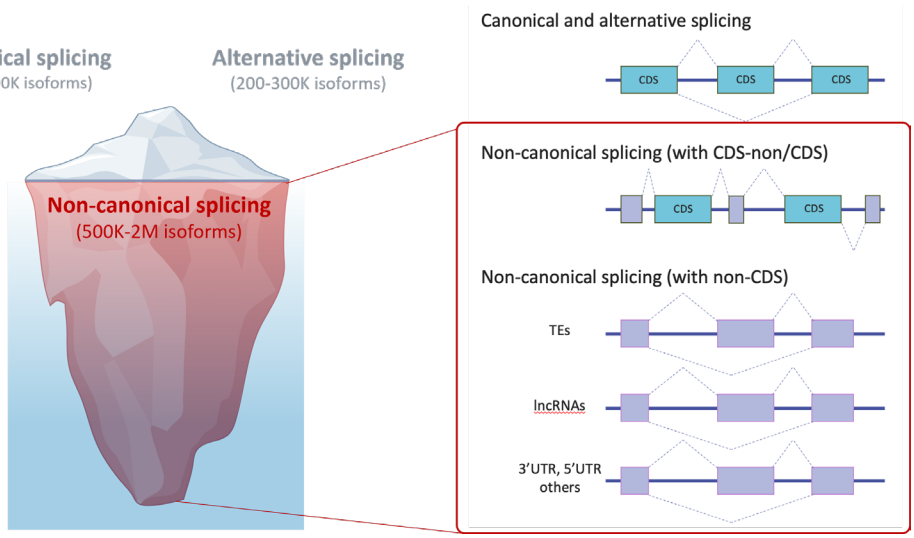

Figure 1: Alternative and non-canonical splicing expand the reservoir for target discovery. *Left side: graphical representation of the transcriptome in the shape of an iceberg, highlighting how non-canonical splicing expands the reservoir of isoforms. Right side: Graphical representation of RNA splicing events (dotted lines) happening in canonical, alternative, and non-canonical splicing. The red square highlights several examples of non-canonical splicing events.*

Non-canonical splicing events have been largely characterised at the transcript level but their contribution to the human proteome remains unclear. Recently, the role of non-coding genomic regions as a source of shared tumour antigens has been investigated. These studies describe the translation of non-coding transcripts which leads to the generation of unique peptides sequences that are tumour-specific and completely absent from conventional annotated proteins (*Attig et al., 2019, Chong et al., 2020, Ehx et al., 2021, Laumont et al., 2016, Laumont et al., 2018; Smart et al., 2018*).

Seminal papers from our founding partner, Institut Curie Centre de Recherche (*Sebastian Amigorena's lab in INSERM-U932*) have further described the non-coding genome as a source for druggable tumour-specific targets (*Merlotti et al, 2023; Burbage et al, 2023; Bonté et al, 2022*):

- In Merlotti et al., the authors discovered that epigenetic alterations in non-small cell lung cancer (NSCLC) result in expression of transposable elements (TEs) and their inclusion within coding-genome-derived proteins through non-canonical splicing, generating thus novel proteins called **Epigenetic Antigens (E-antigens)** (*Merlotti et al, 2023*). These non-canonical junctions between an exon and TE (JETs) are highly tumour specific and shared across patients. Using immunopeptidomics, the authors were able to identify JET-derived peptides presented by HLA-I molecules on tumour cells. Moreover, the JET-derived peptides are immunogenic *in vitro* and specific CD8+ T cells are identified in primary NSCLC tumours and draining lymph nodes. The authors went on to isolate the pJET specific T-cell receptors (TCRs) and to re-express them in primary CD8+ T cells. pJET-targeting CD8+ T cells were then co-cultured with target cells loaded with the corresponding JET-derived peptide and showed specific activation (upregulation of CD137) and target cell cytotoxicity. pJET-targeting T cells were also able to kill target cells naturally expressing the JET-derived peptide (without external peptide addition), demonstrating that the endogenous surface presentation of presented pJETs is sufficient to trigger cytotoxicity. Overall, these results indicate that pJET-specific T cell clones can recognize and kill tumour cells through endogenous HLA-presented pJETs. This is also the first evidence that non-canonical splicing between exons and TEs represents a new reservoir of tumor specific antigens and that they can be targeted by TCR-based therapies, and potentially other immunotherapeutic modalities, such as CARs or TCEs.

- In a parallel study from the same lab (*Burbage et al, 2023*), the authors identified JET-derived antigens in mouse tumour models. Similarly, through immunopeptidomic analyses in tumour cell lines, they identified JET-derived peptides bound to MHC-I molecules. These peptides were immunogenic in tumour-bearing mice. Furthermore, both prophylactic and therapeutic vaccinations with junction-derived peptides delayed tumour growth *in vivo*. These results further suggest that JETs are bona fide tumour antigens, opening possibilities for targeting and vaccination in cancer patients.
- Finally, a third study from the Amigorena's lab (*Bonte et al, 2022*) shed light onto another source of antigens from the non-coding genome only. Combining single cell-transcriptomics, bulk RNA sequencing and immunopeptidomics, the authors identified HLA-I bound peptides encoded by TEs. These TEs are differentially expressed in GBM compared to healthy tissues and cover most tumour cells within one patient tested.

All together these results suggest that E-antigens are addressing the different requirements of an ideal target, setting a new paradigm for antigen-based immunotherapies. At Mnemo, we therefore focus our approach on isoforms derived from alternative and non-canonical splicing, tapping into the majority of the available proteome. Our discovery engine has been uniquely tuned to identify these tumour specific isoforms via the integration of bioinformatics, transcriptomics, proteomics approaches, and functional assays. We have been able to identify two families of targets (**Figure 2**): novel antigenic peptides presented in the context of Major Histocompatibility Complex molecules (pMHC), as well as novel tumour-specific isoforms of transmembrane proteins (TM).

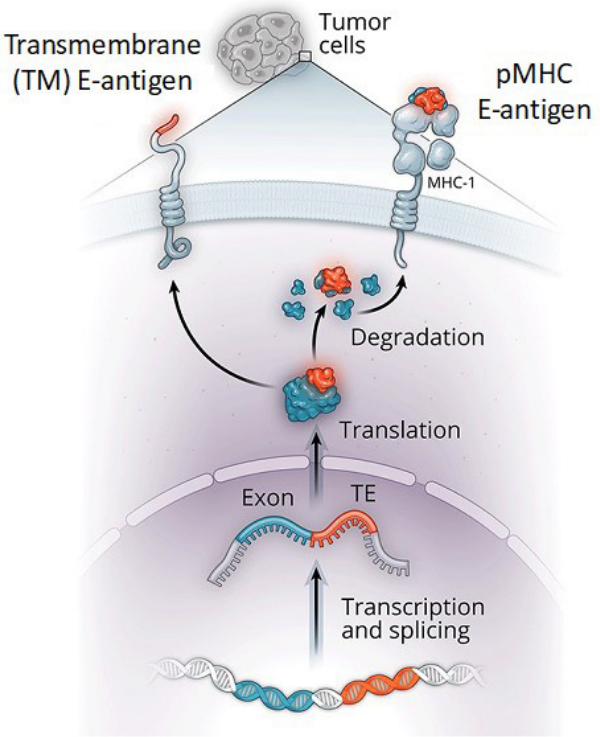

Figure 2: E-antigens occur in two forms, peptides presented on MHC and transmembrane non-canonical isoforms. *Graphical representation of the two families of E-antigens based on the example of junction between exon in blue, and a transposable element (TE) in red. Translation of mature mRNA generates new proteins that are either degraded and presented by the MHC-I complex, called pMHC E-antigen or harbouring a transmembrane domain enabling the presentation of this protein to the cell surface of tumour cell, called TM E-antigen.*

While RNA expression profiles of "conventional" targets (e.g., MSLN or PSMA) exhibit medium to no tumour specificity, the RNA expression profiles of E-antigens demonstrate higher tumour specificity, while keeping high recurrence across multiple indications (**Figure 3**).

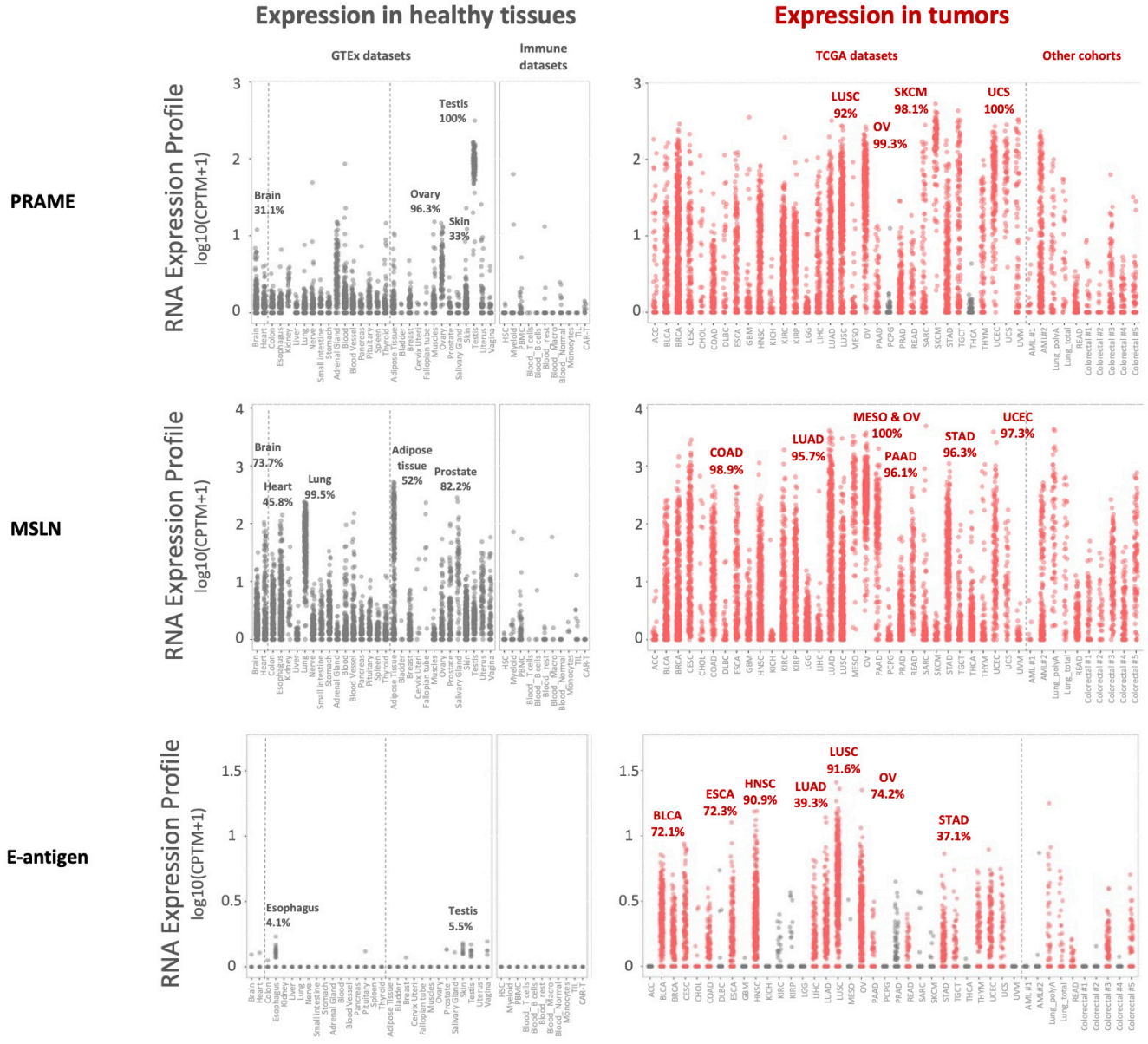

Figure 3: E-antigens show more favourable RNA expression profiles than conventional clinical targets. *RNA expression profile of canonical splicing events of PSMA and MSLN antigens and of non-canonical splicing event of one E-antigen. Public (i.e. TCGA/ GTEx) and in-house bulk RNAseq datasets from healthy (left panel) and tumour (right panel) origins are plotted. Recurrence (%) of specific datasets are also annotated. Expression is normalized to the Count Per Ten Million reads.*

Therefore, by mining alternative and non-canonical splicing isoforms, Mnemo expands the pool of actionable tumour-specific targets. With our proprietary proteogenomic approach, selected E-antigens display all features of an ideal target: high levels of tumour specificity (no/very low expression in healthy tissues), high tumour cell coverage, high levels of recurrence in patients, either within one tumour indication, or across different types of tumours (**Figure 4**).

	Coding Genome		E-antigens
	Point Mutations	Tumor Associated Antigens	E-Antigens
Tumour specific	+	–	+
Tumour coverage	+	+	+
Patient recurrence	–	+	+
Immunopeptidomics (for pMHC only)	–	+	+
T-cell responses in cancer patients (for pMHC only)	+	+	+

Figure 4: *Properties of cancer targets derived from the coding genome only (left part) or from the expression non-coding genome (right part, E-antigens).*

III. Mnemo's target discovery engine: End-to-end discovery pipeline, from target nomination to functional validation.

As introduced in the first part, our integrated proteogenomic approach enables us to discover and validate new tumour specific candidates, either as peptides presented in the context of Major Histocompatibility Complex molecules (pMHC) or as novel tumour-specific isoforms of transmembrane proteins (TM). Our pipeline consists of multiple sequential steps, the first being the identification of target candidates via mRNA expression analyses at bulk and single-cell levels. The next step is to assess whether these transcripts give rise to peptides or proteins that reach the cell surface: 1) for pMHC E-antigens, we investigate whether they are presented on HLA molecules at the surface of tumour cells using immunopeptidomics; 2) for TM E-antigens we examine whether they have the correct topology and surface localisation using algorithm predictions and experimental expression. These steps allow us to robustly identify and nominate candidates with desired features: high tumour specificity and recurrence, as well as correct localisation, and potential druggability (e.g., by identifying accessible epitotes (**Figure 5**)).

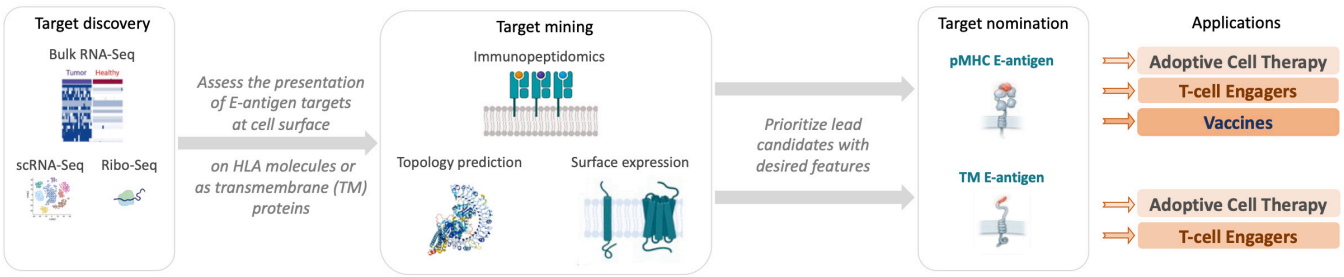

Figure 5: Mnemo's proprietary discovery platform readily identifies E-antigens. *Schematic representation of Mnemo's discovery pipeline showcasing the steps from target discovery, to target mining and nomination, as well as the potential applications of these targets for the development of immunotherapies.*

Once pMHC and TM E-antigen candidates are nominated, the next step is the generation of E-antigen-specific binders, e.g., single-chain Variable fragments (scFvs). Considering accumulated evidence from the aforementioned Institut Curie papers (*Bonté et al, 2022; Burbage et al, 2023; Merlotti et al, 2023*) and in-house Mnemo data (manuscripts in preparation), we have been able to reformat and test E-antigen binders in multiple ways: IgG, CAR, TCE (**Figure 6**). Mnemo's team has developed expertise in a diverse set of immunotherapy modalities that cover the full range of antigen densities, such as CARs and TCEs and including ultra-specific TCR-like antibodies (i.e, MHC-restricted binders). Therefore, for binder screening and validation, we perform in-house iterative deep screening which identifies the optimal binder and therapeutic modality for each E-antigen target. This includes functional screening of the binders, biological profiling, modality prioritisation, and, finally, *in vivo* validation. Moreover, full IgGs, enable us to validate the presence of the E-antigen target itself via antibody-based detection by microscopy. This parallel approach of target expression validation and functional lead binder validation allows us to accelerate the path to preclinical validation. For pMHC E-antigens, we are also building a large peptide bank, composed of peptides detected by immunopeptidomics in tumour samples and derived from tumour-specific junctions, which can be leveraged for tumour vaccine design.

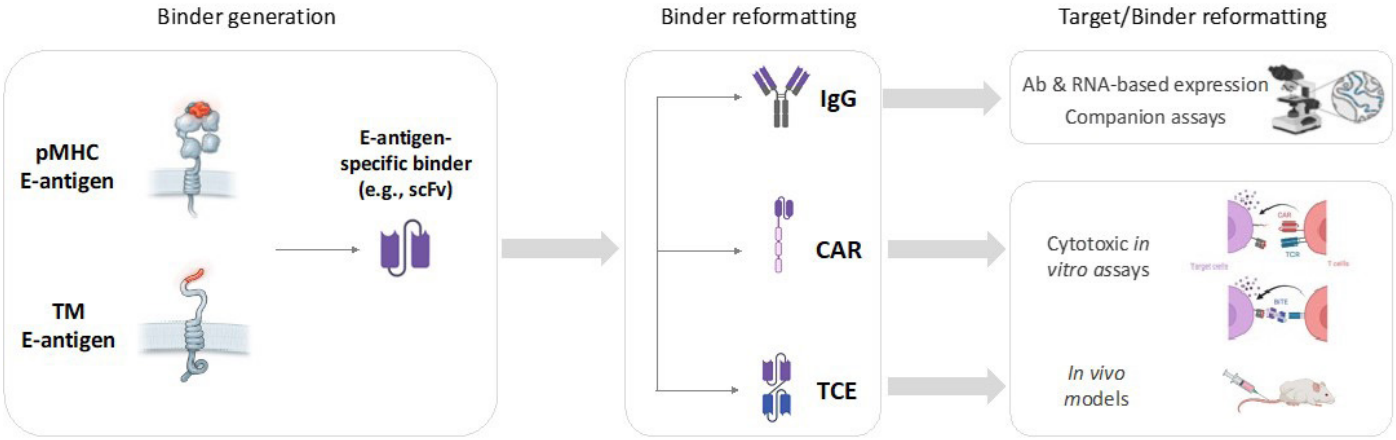

Figure 6: Mnemo's validation platform for E-antigen-specific binders. *Schematic representation of Mnemo's validation pipeline from binder generation to reformatting and validation.*

Conclusion:

At Mnemo, we have identified a novel class of tumour targets, which we call **Epigenetic Antigens (E-antigens)**. These antigens, derived from the expression of non-coding genome, occur in two forms (pMHC and TM). They exhibit high tumour specificity and recurrence, making them promising candidates for next-generation cancer immunotherapy. We have built an in-house pipeline with robust methodologies for end-to-end target discovery and validation. Using multiple *in vitro* and *in vivo* models, we have demonstrated that E-antigens are druggable by multiple immunotherapy modalities such as CARs, TCRs and TCEs, and represent an actionable target reservoir for cancer vaccines. In summary, Mnemo has developed an integrated discovery and validation engine for novel targets that can set a new paradigm for cancer immunotherapy.

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TOWARDS A NEW GENERATION OF PRECISION MEDICINE IN IMMUNO-ONCOLOGY, WITH PROPRIETARY STC PLATFORM, DESIGNED TO GENERATE FIRST-IN-CLASS CANCER VACCINE

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1. Immuno-oncology treatments: from challenges to solutions; an urgent need to anticipate tumor's evolution and overcome immune evasion.

Cancer is a moving target: it constantly adapts and evolves to escape treatments. This ability makes it one of the most challenging diseases to cure and the second leading cause of death worldwide. In 2022 alone, 20 million new cases and 9.7 million deaths were reported globally. (1) Particularly for patients with advanced or metastatic cancer (representing 25% of newly diagnosed patients), the current arsenal of treatments is often inefficient. This is mainly because tumor cells adapt and become resistant to these therapies. This high adaptability leads to treatment failure in 90% of patients with solid tumors, which affected 16 million patients in 2022(2). There are two key factors driving this consistent drug resistance:

- The multiple and evolving antigens expressed by tumor cells, result in the inaccuracy of the targets addressed by current treatments.
- The capacity of tumor cells to evade the patient's immune system requires a more effective education of the immune system.

In the last decade, highly innovative approaches such as immunotherapies have revolutionized cancer treatment by bypassing the evasion strategies of malignant cells and boosting the immune system against cancer. Anti-tumor immunotherapy, such as immune checkpoint inhibitors (ICIs), is now known as an effective approach for treating cancer. However, the low immunogenicity of certain tumor cells often allows them to escape the immune system. Only 15% of patients are eligible, and at best, half of them respond to these treatments. Therefore, developing new strategies, has become a crucial goal in immunotherapy to enhance immunogenicity and efficacy in a wider range of patients.

Colorectal cancer (CRC), for example, is the third most common cancer worldwide, with 2 million new cases annually. It is also the second leading cause of cancer-related deaths, resulting in 1 million deaths each year worldwide (3). Despite advancements in screening that have reduced both incidence and mortality rates, many early-stage CRC patients (25% to 50%) will eventually develop metastatic disease (mCRC) (4). There are two main categories of mCRC:

mCRC Population	Description of tumor types	Current therapeutic solution and survival probability
dMMR MSI-H: 5% of mCRC population	Tumors with deficient mismatch repair (dMMR) and high microsatellite instability (MSI-H) are described as "hot" tumors. They exhibit a high tumor mutation burden and have more tumor-infiltrating lymphocytes. (TILs)	Immunotherapies such as immune checkpoint inhibitors (ICIs) have been found to be active for dMMR /MSI-H mCRC in enhancing the activity of immune cells against tumors (Anti-PD1, PD-L1).(5–8) However, only 48% of patients will respond to ICIs treatment and 1 out of 2 patients will experience early progression after 3 months of treatment.(4)
pMMR MSS: 95% of mCRC population	Tumors with proficient mismatch repair (pMMR) are characterized by microsatellite stability (MSS) and are described as "cold" tumors without immune cell infiltration the tumor microenvironment.	The majority of mCRC patients are not sensitive to immunotherapies (9) and are still treated with standard-of-care chemotherapies. While effective, these treatments often come with toxicities that make long-term administration difficult, increasing the risk of cancer recurrence due to the well-known progression and resistance mechanisms of tumor cells under therapeutic pressure. (10,11) The five-year survival rate remains below 12% (1)

Table 1: *mCRC patients population overview*

Therefore, there is a high unmet need for a disruptive treatment capable of combating immunotolerance and resistance to standard treatments, significantly improving patient outcomes.

Various innovative anti-cancer vaccine approaches are in development, such as mRNA, protein, and peptide strategies (12,13). These therapies have shown promising clinical results with positive safety data in solid tumors (14), but they are still limited in terms of the panel of antigens presented to the immune system, most of which are not linked to tumor plasticity and relapse mechanisms. Additionally, these types of autologous and personalized approaches present manufacturing challenges that may be a barrier for patient access, particularly when a patient-specific biopsy is required and could be time-consuming.(15)

Given the lack of effective therapeutic solutions for these patients, there is a pressing demand for accessible innovative drug candidates capable of anticipating and overcoming tumor plasticity and immune escape mechanisms.

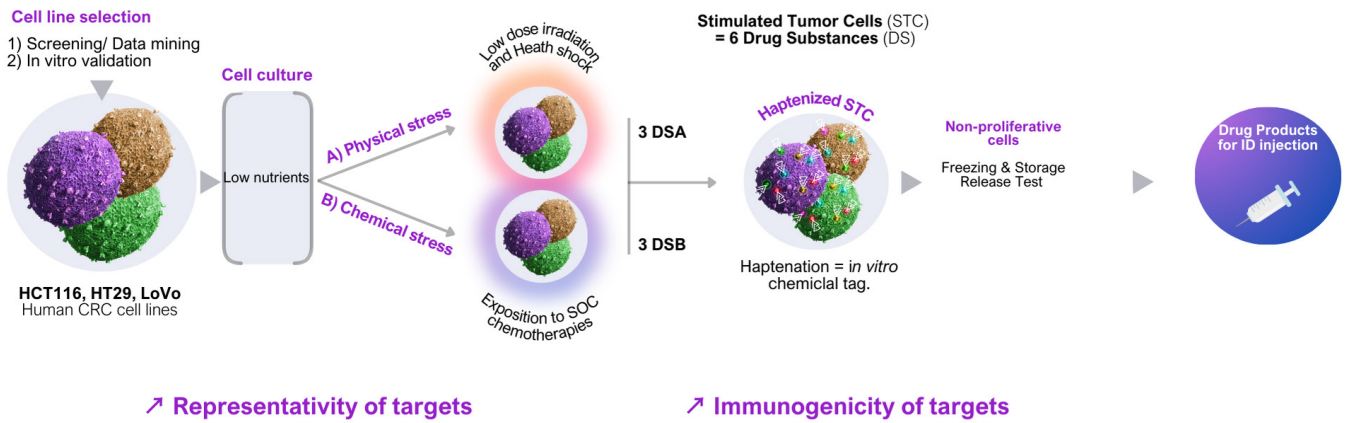
2. Brenus STC "Stimulated-Tumor-Cell" platform: an innovation mimicking patient's relapsing condition and educating immune system to anticipate tumor progression.

To address this unmet need, Brenus Pharma, a French biotechnology company based in Lyon, has developed an innovative and proprietary platform called Stimulated-Tumor-Cells (STC). STC technology not only fights cancer as it is today; but also anticipates how it will evolve in the future. It trains the patient's immune system to recognize and destroy tumors, regardless of their evolution.

This platform is designed to produce "off-the-shelf" and "first-in-class" therapeutic cancer vaccines with the ambition of simplifying and standardizing vaccine manufacturing.

Our platform generates innovative "Stimulated-Tumor-Cells" vaccines (STCs) in 4 steps (Figure 1):

Figure 1: *STC Technology design and mode of action.*



STEP 1) TUMOR CELL SELECTION	STEP 2) STIMULATION PROCESS	STEP 3) CHEMICAL TAGGING	STEP 4) GHOST CELLS PROCESS	What is expected to happen after vaccine injection
The raw material identification process is supported by advanced computational multi-omics analyses and patient biopsy databases, providing a robust methodological framework. *Selection of allogenic tumors from cell banks, based on:* • *Targeted indication* • *Resistance profile* *Large stock defined and characterized.* **STC-1010 vaccine:** • *HCT-116, LoVo (both MSI-H) and HT29 (MSS).* • *Covers both cold and hot CRC tumor phenotypes.* • *Harbors specific codants and non-codants neoantigens identified from Cellines.tron.* • *Covers key oncodriver mutations shared with >5000 CRC patient's tissues. (Figure 2A)*	*After selection, the cells are exposed to standards of care stimulating real conditions in patient relapse. This process induces the overexpression of tumor antigens, related to therapeutic pressure and leading to tumor plasticity. By replicating the conditions encountered by patient tumor cells, the STC platform ensures the production of vaccines that effectively mimic the adaptive responses of tumors.* *This standardized process (Figure 2B) provides a significant technological advantage by enabling access to new classes of therapeutic targets for the immune system.* *Expression of targets is guided by proteomics.* **STC-1010 proteome:** • *Shared 200+ tumor antigens from cancer-related proteins (CRP) identified from the Human Atlas protein's database (incl. 6000 patient's biopsies).*	*A further in vitro step of haptenation is performed to conjugate chemical sequences (haptens) to STCs, creating a strong covalent bond. (16).* *This hapten conjugation (haptenation) aims to enhance the immunological potential of STCs boosting STC recognition by antigen-presenting cells (APC). (17)* *Haptenation has been proven to be an efficient strategy to safely enhance the immunological function of vaccines (showing a 5-year overall survival rate of 44% in 214 patients, with no toxicities reported).(17)*	*Stimulated and haptenated tumor cells are inactivated to create "ghost cells."* *This inactivation process ensures that the cells are safe for use while retaining their ability to provoke an anti-tumor immune response:* *These cells are non-proliferative but maintain an intact biological membrane, preserving the essential antigenic properties necessary for immune system recognition and education.*	*1. Maturation and activation of Antigen-Presenting-Cells (APCs) generally Dendritic Cells (DCs)* *STC internalization by APCs (No HLA restriction)* ↓ *2. Cross Presentation: T-cell priming and activation by matured APCs.* ↓ *3. Expansion of the selected multi-specific T cell population* ↓ *4. Destruction of patient's tumor cells* *Possible synergy with immune checkpoint inhibitor.*

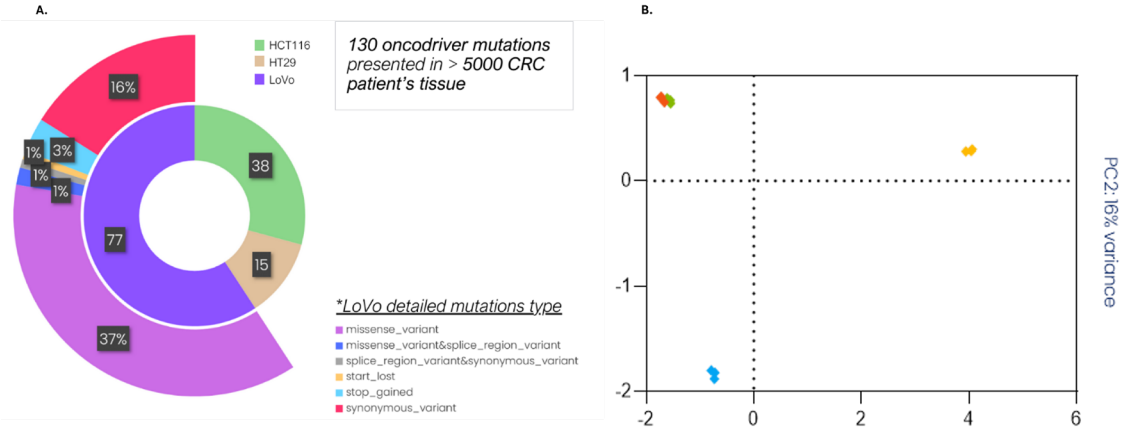


Figure 2: STC-1010 characterization. *(A). Whole exome sequencing (WES) of STC-1010 cell lines: HCT116, LoVo (MSI-H) and HT29 (MSS), with LoVo detailed mutations type*. 130 (77+38+15) oncodriver mutations shared with more than 5000 CRC patient's tissue from http://coloncanceratlas.org/ (Including BRAF, KRAS, TP53...) (B). Principal Component Analysis by RNAseq on 3 STC-1010 batches (RD1, RD2, RD3) reveals a specific transcriptomic signature linked to the application of each stress, with perfect reproducibility batch to batch. Orange: RCB Raw Cell Bank, Green: MCB Master Cell Bank (RCB + serum depletion), Blue: DSA Drug Substance with physical stimulation, Yellow: DSB Drug Substance with chemical stimulation.*

Thanks to this innovative protocol, we educate the immune system to detect a broader range of cancer targets including those responsible for cancer's resistances and escape. The STC approach not only addresses the immediate threat but also arms the immune system against tumors that might appear later, significantly reducing the risk of relapse.

The STC platform is adaptive, capable of targeting over 200 cancer-related antigens; efficient, with a scalable off-the-shelf production process that ensures swift availability to those in need; and innovative, leveraging a deep understanding of cancer's complexity to offer a new kind of precision treatment that's not just reactive, but proactive.

The first candidate, STC-1010, targets colorectal cancer (mCRC), and presents strong advantages overpassing the limits faced by current vaccine strategies thanks to an innovative bioproduction process.

	Personalized cancer vaccine strategies	**Brenus PHARMA STC-1010 vaccine**
Access to treatment	Invasive: tumor biopsy needed, HLA restriction.	**No tumor biopsy needed, No HLA restriction.**
	• *Due to often limited access to the tumor or the disseminated metastases, a high number of patients (60-70%) cannot be operated to have a biopsy, and thus can't access the treatments, where the biopsy is necessary. Compared to this, no tumor biopsy is needed with STC.* • *Absence of HLA restriction: Compared to whole cells, dendritic cells, and peptides vaccines, the STC-1010's allogeneic approach enables it to escape HLA restriction and activate the MHC-1 complement pathway of the patient's own immune cells, making the treatment available to a wider population.*	
Repertoire of targets / Targets selection	Limited / Validation based on gene extrapolation. (NGS platform)	**Mimicking patient's mechanism of relapse to increase panel of targets. Validation based on proteome. (LC/MS)**
	• *STC targets validation is based on the protein and not the gene, allowing for higher accuracy.* • *Broad repertoire of antigens presented: Compared to mRNA, DNA, Peptide, Dendritic cells vaccines (which present up to 34 Ag), STC-1010 is the therapeutic solution with the widest panel of antigens of interest: proteomic studies of the product, compared with large, published databases derived from patient biopsies, have shown the systematic presence of 200+ cancer-related antigens of interest in the final product.*	
Immunogenicity	Potential safety issues with viral vectors.	**Enhanced by haptenation and ghost cells, maintaining safety.**
	• *Enhanced immunogenicity: haptenation, and production of "ghost cells" (apoptotic non-proliferative cells with an intact membrane), and the use of a low-dose immunostimulant, enhance STC-1010's immunogenicity by ensuring effective education of the immune system, thereby surpassing the limits of other approaches (mRNA, DNA, peptide, and whole cell vaccines) while maintaining product safety. These technologies allow to avoid the use of "viral vectors", suspected of safety issues by FDA.*	
Supply / Manufacturing	"Patient / Patient" Complex, long and costly.	**"Off-the-shelf" standardized manufacturing, Saving cost and time.**
	• *Manufacturing process: We have a great advantage on complex, long and costly production process of the CAR-T cells, and other personalized therapies. The expertise of our co-founder, B. Pinteur, in the bioproduction of immune-therapeutics, combined with a risk analysis carried out by recognized companies, has enabled us to develop standardized processes, transposable to large-scale manufacturing (GLP, GMP, scale-up) right from the early stages of development. This will guarantee excellent control of costs and production times.* • *STC Platform is standardized and ready-to-use. Current production capacity for the clinical trial: 1 batch of 800 doses in less than 3 months (80 patients treated over a year). Possibility of parallelizing the batch production.*	

Table 2: *STC-1010, a unique value proposition anticipating tumor's evolution.*

3. Demonstrating the preclinical effectiveness and clinical potential of STC-1010 to treat colorectal cancer patients – BreAK CRC Study.

STC-1010 has demonstrated its innovative potential and effectiveness in pre-clinical trials, showing promising results, published in renowned international congresses, in varied tumor models and clinical conditions.

The vaccine leverages the unique advantages of the STC platform, including the robust selection of tumor cell lines, simulation of relapse conditions, and enhanced immunogenicity through haptenation and inactivation techniques.

<table>
<tr><th colspan="4">1. PRODUCT CHARACTERIZATION</th></tr>
<tr><td colspan="2">RNA sequencing of STC-1010 cell lines
HCT116, HT29 & Lovo (CRC cell lines)</td><td colspan="2">Genomic, Transcriptomic, Proteomic &
Surfaceomic analyses</td></tr>
<tr><td colspan="4">Brenus Pharma's expertise in proteomic analysis and patient data correlation ensures that the STCs accurately reflect the CRC clinical heterogeneity.</td></tr>
<tr><th colspan="4">2. HEALTH AUTHORITIES' PRECLINICAL PACKAGE
Validate Safety & Efficacy in Gold standard CRC models for 1st Line approval</th></tr>
<tr><td>POC 1 - in vivo
Syngeneic mice bearing CRC tumor treated with 1 cell line STC-1010 surrogate.</td><td>POC 2 - in vivo
Syngeneic mice bearing CRC tumor, treated with
3 cell lines surrogate = mSTC-1010.</td><td>POC 3 - in vivo
Syngeneic mice bearing Anti-PD1 Resistant tumor model treated with mSTC-1010.</td><td>POC 4 - in vivo mSTC-1010 + Standard Of Care association</td></tr>
<tr><td colspan="4">Efficacy & Safety: Demonstrated tumor volume reduction and improved survival in different murine in vivo models, combined with immunostimulants in low doses associated or not with the standard of care (SoC) chemotherapy (FOLFOX or FOLFIRI) with no safety issues</td></tr>
<tr><th colspan="4">3. EXTRAPOLATING HUMAN CONDITION
Validate MoA, Safety, Efficacy & Comply with FDA's Modernization Act 2</th></tr>
<tr><td colspan="2">CAM= Chicken embryo model (chorio allantoic membrane) - in ovo Potency test using human STC-1010</td><td colspan="2">Immune response activation (MLR derived model) and induction of tumor killing - ex vivo
Potency test using human STC-1010</td></tr>
<tr><td colspan="4">Mechanism of Action: Enhanced T cell activation and infiltration into the tumor via pro-immune cytokine secretion, leading to massive apoptosis of allogeneic CRC tumor cell lines, tumor necrosis and reduction of metastasis in ex vivo and in ovo models extrapolating human condition.</td></tr>
</table>

- G. Alzeeb et al, Front. Oncol.Sec. Cancer Immunity and Immunotherapy 2024, DOI: 10.3389/fonc.2024.1427428
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- F. Ghiringhelli et al, Cancer Res 2024https://doi.org/10.1158/1538-7445.AM2024-5003 Link to Poster
- B. You et al, Clinical Oncology Journal 2024DOI:10.1200/JCO.2024.42.16_suppl.TPS3635 Link to Poster

Based on STC-1010's robust validated preclinical package including in vivo, in ovo, and ex vivo studies (Table 3), a first-in-human phase I/IIA ("BreAK-CRC") study will be launched in 9 oncology early phase excellence centers in immuno-oncology with expert investigators in immunotherapy.

The "BreAK-CRC" trial protocol has been reviewed in a pre-submission meeting with the French National

Health Authority, and the submission of the CTA (Clinical Trial Authorization) through the clinical trial information system of the European Union is ongoing.

BreAK-CRC" is meant to clinically confirm the promising preclinical outcomes obtained on different syngeneic mouse models and ex-vivo assays about the potential major therapeutic impact of STC-1010 on patients with pMMR/MSS and dMMR/MSI-H ICI-resistant advanced or metastatic unresectable colorectal cancers.

The Phase I/IIA clinical trial, aims to evaluate the safety and efficacy of STC-1010 in patients with unresectable advanced or metastatic colorectal cancer. The phase I will assess the tolerability of different dose levels of STC-1010, combined with low-dose immunostimulants and standard of care chemotherapy. Following this, the Phase IIA will further evaluate the treatment's efficacy, particularly focusing on 12-month progression-free survival rate. Exploratory analysis will evaluate the immune response and the ctDNA dynamic.

Study beginning is planned for the end of 2024:

- Phase I safety readout ≈ Q3 2025,
- Phase I efficacy and safety results ≈ Q3, 2026
- Phase IIA main results ≈ 2029

4. Strategic partnership with the French Blood Establishment (EFS) for clinical bioproduction.

In 2023, the French Blood Establishment (EFS) and Brenus Pharma announced their strategic partnership for the STC-1010 clinical bioproduction. This collaboration is part of a shared vision: developing the French bioproduction sector nationally and internationally.

EFS' Cell Therapy and Engineering Unit (UTICELL), based in Saint-Ismier, France, has demonstrated recognized expertise in the cell therapy field since 2003 confirmed by an European GMP Certification issued by the ANSM in 2015 for manufacturing, control, and storage sites for ITDs (Innovative Therapeutic Drugs). Both teams have been collaborating effectively for several months to produce GMP clinical batches through an initial scale-up.

"*This collaboration reflects the importance of having both technical and scientific expertise in France to quickly advance the development of this promising drug and fulfill our public health mission. From the first exchanges with Brenus, we were impressed by the maturity of the STC1010 project and convinced that through this collaboration, we could give patients a chance to fight their cancer.*" Anaick MOISAN, Pharmacist Delegate, UTICEL EFS Rhône-Alpes.

5. Leveraging our STC Platform: the technological relay to effectively treat solid tumors and avoid cancer recurrence.

The STC vaccine, based on the stimulation of ghost tumor cells guided by proteomics, has demonstrated significant preclinical anti-cancer effects. Proof-of-concept studies have confirmed the potential of the STC approach to treat CRC pMMR/MSS or anti-PD-1 resistant cancers by effectively educating and activating the immune system.

Moreover, thanks to a standardized 'off-the-shelf' approach and CMC expertise reinforced with a strong manufacturing partner, STC vaccine production is more time-efficient and cost-effective compared to other innovative cancer vaccine strategies, making it more accessible for patient with unmet needs.

Additionally, the STC platform stands out as a major technological relay for the pharmaceutical industry

in immuno-oncology.

This is made possible by a "ready to use," standardized, and patented platform (IP protection: 1 International patent covering the STC platform, and product patent covering the vaccine generated, coverage across 22 key markets .) that offers complete flexibility in targeting various types of cancers with patented products. The platform facilitates the quick generation of new STC treatments to meet other patient's needs in diverse solid tumors indication. (Ovarian, Lung, Pancreas…)

The need for new approaches is especially crucial as pharmaceutical companies must combine their leading immuno-oncology drugs which are approaching the end of their patent protection within the next seven years. Brenus Pharma is now anticipating scientific and clinical collaboration models which aimed at synergizing pipeline development in immuno-oncology.

These models include:

- A clinical combination strategy for the first product in mCRC and other gastric cancers (with Immune Checkpoint Inhibitors/Antibody-Drug Conjugates).
- Co-development of the second asset in ovarian cancer, (STC-1020 in R&D development phase)
- Tailored co-development for new assets aligned with strategic indications.

In conclusion, current trends in immuno-oncology indicate that most licensing deals are made early at the end of the preclinical phase or after Phase I preliminary results (18). This dynamic, proactive approach positions Brenus Pharma to effectively meet the evolving needs of the oncology market.

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ACCELERATE ANTIBODY DEVELOPMENT THROUGH QUANTITATIVE SYSTEMS PHARMACOLOGY – THE PEMBROLIZUMAB STORY

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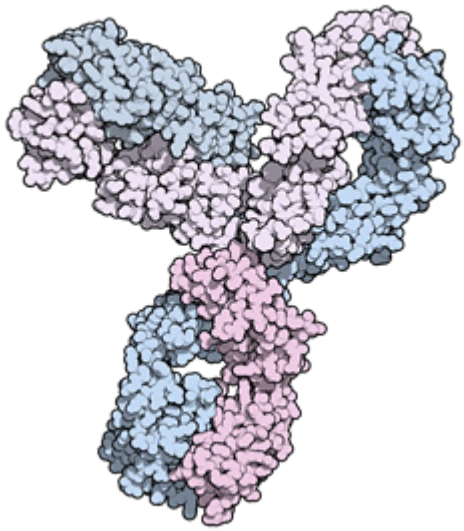
Take home messages

Drug development was radically accelerated using quantitative pharmacology approaches as per the pembrolizumab story. Everything becomes fluid under pressure, as is exemplified by new modeling approaches, clinical paradigms and regulatory pathways that were applied to bring this PD-1 inhibitor to the market. Last but not least, personal motivation and sponsorship are also important, as the paths of drug development are not always straightforward. Hurdles present themselves along the way, requiring significant efforts in order to succeed.



Monoclonal antibody	
Type	Whole antibody
Source	Humanized (from mouse)
Target	PD-1
Clinical data	
Trade names	Keytruda
Other names	MK-3475, lambrolizumab
AHFS/ Drugs.com	Monograph
MedlinePlus	a614048
License data	US DailyMed: Pembrolizumab
Pregnancy category	AU: D[1]
Routes of administration	Intravenous
Drug class	Antineoplastic agent
ATC code	L01FF02 (WHO)

Figure 1: *Pembrolizumab. The humanized antibody pembrolizumab used for cancer immunotherapy that treats several cancer types which targets the programmed cell death protein 1 (PD-1) receptor of lymphocytes ("Pembrolizumab," 2024).*

Introduction

The medicine Keytruda almost needs no introduction. It is the PD-1 inhibitor marketed by MSD, or Merck & Co in the USA, which is active in a wide range of indications in solid tumors and beyond. Pembrolizumab is currently the best-selling drug worldwide, with a forecast of over 27 billion dollars for 2024 (Verdin, 2023) . It is a human recombinant antibody drug of the IgG4 class and has to be administered intravenously. Whereas many modern oncology drugs are active in a limited set of indications, pembrolizumab is active in an ever expanding range of indications and combinations (figure 1).

Pembrolizumab acts by blocking the naturally occurring ligands PD-L1 and PD-L2, as they are expressed on normal cells, from binding to the PD-1 receptor that is expressed on predominantly T-cells. Activation of the receptor is involved in regulating immune response. Regulation is needed to keep the immune system in check – you would not want an otherwise useful immune response to last forever and eventually attack all cells in your body. The PD-1 pathway is one of the brakes of the immune system. Unfortunately, this also has been learned by tumor cells: there are many cases in which cancer cells are able to escape the immune system by over-expressing PD-L1 and hijacking the brake. We as a scientific community have still not figured out what exactly the circumstances are under which it happens.Pembrolizumab prevents

the activation of the PD-1 pathway, releasing the brake and thereby re-activating the immune system to attack cancer cells (Hamid et al., 2013).

Quantitative Systems Pharmacology (QSP) is the scientific discipline that relates dose to concentrations in the body over time and the resulting effects as they develop in various conceptual stages, e.g. at the biochemical, the cellular or the tissue level. This paper describes the chronology of development of pembrolizumab from the perspective of clinical pharmacology and QSP, related to dose selection, dosing regiments and efficacy-safety evaluations, highlighting opportunities that can be used also in the development of new drugs.

Acknowledgements

The author was employed at Organon, Schering-Plough and MSD in the period from pembrolizumab's inception until the initial drug approval and was part of the R&D team in various stages. All the information in this paper can be traced back to public sources. The author is an inventor of a –peripheral– patent of pembrolizumab (Carven et al., 2012) and has co-/authored nine papers on the subject, published in, among others, the Lancet, NEJM and Clinical Cancer Research. Many intricacies of the story have been left out; the reader is referred to other publications for intriguing details on how the project evolved in the first years.

QSP models can describe biology at different levels of detail. In this paper, the term is interpreted broadly and covers the range from mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) modeling to models that are more related to systems biology.

The beginning

The development of pembrolizumab started as an immunology project in 2002 (Shaywitz, 2017), a therapeutic area utterly different from oncology. The project was run at a company called Organon, which has recently been reactivated with a focus on women's health, but at the time, was an international pharmaceutical company headquartered in the Netherlands. For the target to work in immunology, agonistic antibodies were required. Agonistic antibodies against PD-1 would engage the brake, and stop the overly high activity of T-cells in auto-immune diseases. The team was not able to recover such antibodies with sufficient potency. Antagonistic antibodies were, however, developed, one of which would become MK-3475 later on. Immuno-modulation as a potential treatment in oncology was obscure. The antagonists were not deemed as valuable but could still be worthwhile in anti-infective treatments such as antiviral indications (figure 2). The project was allowed to continue with the most potent antagonists (Shaywitz, 2017).

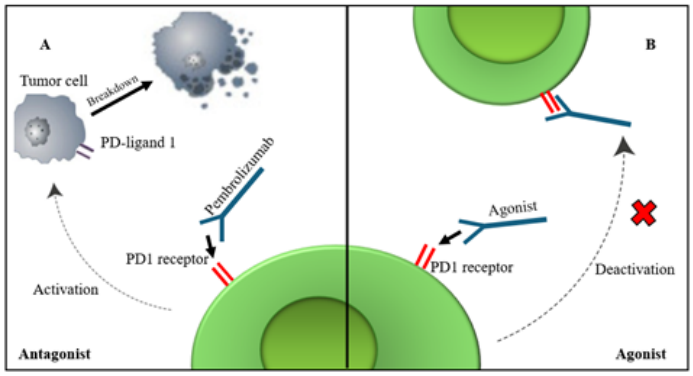


Figure 2: *T-cell activation and deactivation. The T-cell can get activated by antagonist pembrolizumab binding to PD1 receptor, this will result in tumor cell breakdown. If an agonist will bind to the PD1 receptor of a T-cell, it deactivates the T-cell.*

Second life

New project members were engaged, and a plan was set up that focused on an ex-vivo blood immuno-stimulation assay to evaluate different diseases and indications. The assay was evaluated in an early study in cynomolgus monkey, chosen because it was the only species with similar binding potency as for humans (Carven et al., 2012). It was not overly clear which indication would benefit the most from PD-1 modulation, and the program was considered to have limited priority. This almost led to the cessation of the project. One scientist in the company, Andrea van Elsas, sponsored the program as he was aware of the potential benefit in oncology, which led to a pivot in 2006 (Shaywitz, 2017), an example of the aforementioned hurdles and the sponsorship that is subsequently required to overcome them. Such need at this particular injunction moreover foreshadows further evolution of events.

Organon was acquired by Schering-Plough for 14B$ in 2007, which led to a pause, given the priority of the program (Biospace, 2007). The data package that was built in combination with the strong sponsorship however appeared sufficient. The exposure-response (E-R) curve, as determined by modeling, was presented, figure 3, critically providing the new leadership with the assurance of the ability of the team to understand and control the clinical pharmacology of the compound. An early development team was created and prepared for first-in-man entry with various activities, such as a repeated dose study in monkey, following mostly standard paradigms. An update of the model using the new cynomolgus data was performed but it had limited impact.

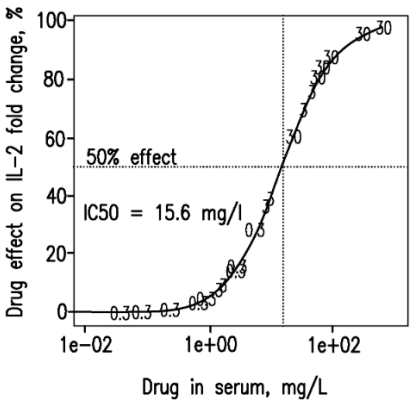


FIG.10B

Figure 3: *Exposure-response curve by PK-PD modeling for pembrolizumab in monkeys (Carven et al., 2012).*

Another outside event that influenced the program was when MSD acquired Schering-Plough for 42B$ (Teather, 2009). The program was low in priority – l'histoire se repete. MSD HQ announced it would close the R&D site in the Netherlands (Munafacturing Chemist, 2010), through which sponsorship was lost, and the program was even put on a list to be out-licensed (Shaywitz, 2017). The project was hanging by a very thin thread. A small contingent of development was kept in Oss later in 2011, including a group of QSP modelers (Pharmafile, 2011).

Third life

Immuno-oncology was still an unknown field when, in 2010, the competitors at BMS announced small but meaningful effects of ipilimumab in solid tumors. Roughly one out of ten patients responded with a tumor decrease but with durable responses. Interest in the shelved project was rekindled, and it progressed towards human in its second resurrection. A complete set of data, including pharmacokinetic (PK) and pharmacodynamic (PD) sampling, was available by the end of 2011. Several assays were attempted for

PD markers, but only the ex vivo assay, the same as earlier applied in monkeys, yielded usable albeit variable results. Modeling support was resumed by the author, focusing first on pharmacokinetic topics such as dose linearity and comparability to the similar PD-1 inhibitor, nivolumab, from BMS, for which some early results were published. While the clinical group expressed interest in deviations from dose proportionality, as was clear from the first escalation data, experience across early programs also contributed to a different assessment (Patnaik et al., 2015). Reduced exposure in the lowest dose group might also have been due to typical variability instead. Modeling of both PK and PD allowed the team to also discuss topics such as selection of potential efficacious doses and dose interval. The first cohorts of the study were executed as bi-weekly dosing. Using modeling it was clear from the beginning that the coverage in blood would not be meaningfully lower with dosing every three weeks (Q3W), a regimen more compatible with drugs already on the market. A QSP modeling strategy was drafted to cover these topics and more; eventually, all of them would be executed. No further resurrection of the program was needed; in this case, three lives were enough.

The program reached higher priority, more attention from management, and several team lead rotations. The same applies to members – almost nobody stayed from 2011 to the initial filing. Meanwhile, the decision was made to expand the cohort in advanced melanoma, and by mid-2012, the results were shown internally at a face-to-face team meeting (Shaywitz, 2017). They were so excellent that people literally cheered. Half of the patients responded to the drug, in a disease that killed most people in a year at the time. This event marked a new beginning for the program. It was no longer an obscure project in the cellars of the portfolio. Academic researchers and management alike were convinced, and the project got the highest priority. Everything the team needed would be provided. At the same time, the team was also given a daunting task: the 2+ years we were behind on BMS because of all the hesitation and portfolio discussion were to be gained back. MK-3475 would not stand a competitive chance otherwise. The challenge posed also provided an opportunity for the discipline of QSP modeling, as it is possible to derive knowledge from clinical trials rather than slowly executing separate clinical trials, as was the classical paradigm. In addition, it was decided to expand the original first-in-man trial, later known as KEYNOTE-001, rather than starting new trials to save time. Earlier in the year, a cohort of patients at Q3W at the same dose of 10 mg/kg and a lower dose of 2 mg/kg Q3 was selected based on modeling of still of the same ex-vivo biomarker.

During this period, a lot was happening in parallel. It became evident to all involved that the ex-vivo biomarker was useful but did not provide a comprehensive understanding of what happens in the tumor. To bridge this gap, a series of deep dive meetings were set up, including attendance from a research pharmacology group in Palo Alto led by Joe Philips, which would be the basis for our later modeling effort (Lindauer et al., 2014). The philosophy behind QSP modeling is that the conceptual steps between dose and effect are tracked and captured by models, see Figure 4. Classical pharmacology relates dose levels to observed efficacy or safety. QSP modeling aims to describe what happens in pharmacology over time and dose. One needs to chart the individual steps in the cascade between dose and effect and define the critical steps. Drug binding to its target is an example of a critical step and forms the interface between pharmacokinetics and pharmacodynamics. Early experiments showed that T-cells expanded upon dosing to tumors. On top of that, the density of receptors was also increased. These and other deliberations clarified the experimental data needed to enable proper QSP modeling. Therefore, while we were already well into the clinic, a series of difficult and laborious animal research experiments were set up, including the ‘mousification’ of the drug. While this was set in motion, more modeling efforts were initiated in parallel, such as tumor size modeling.

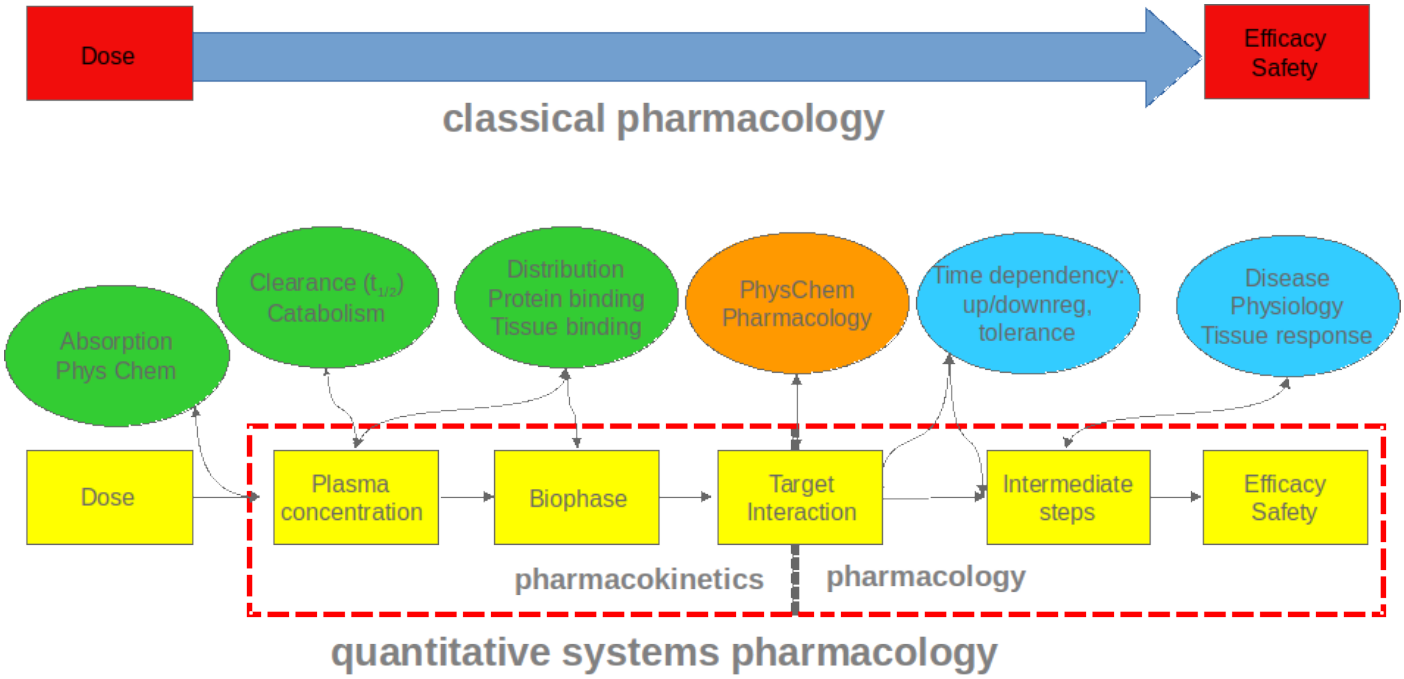


Figure 4: *The conceptual steps followed by QSP modeling, with a target binding as the interface between pharmacokinetics*

It is all about the dose

The clinical data from the expansion cohorts became available in the last trimester of 2012. The results of the lower dose cohort were disappointing, with the response rate more or less halved. With hindsight, it is clear what was going on, but right then the question was: "What happened to 2 mg/kg?". It was advocated to stop the study of the low doses, which almost happened. The modeling of tumor size over time was intensified, and by the end of the year, it clearly showed that there was no difference between the dose levels in relation to the speed of tumor size change. The result was even more puzzling, as it was unclear where the difference was coming from. A decision was made in a crucial small meeting with senior management by the end of 2012 that modeling results warranted further scrutiny, and the low dose level was not canceled.

The default paradigm in oncology was the so-called 3+3 design to find the maximum tolerated dose, i.e. the dose that does (just) not kill the patient but hopefully kills the tumor. The default perspective was quite orthogonal to the concept of further evaluation of the low dose. Therefore, the position on the low dose was heavily debated throughout the team and management layers in the following period. It was also acknowledged that the sequential recruitment in the progressively lower-dose cohorts could have induced bias (Hamid et al., 2013). And indeed, a clear difference was found in the cohorts, as the baseline tumor size was increasing with the cohort. Baseline tumor size was, perhaps counter-intuitively, not recognized as a prognostic marker in advanced melanoma and therefore, the argument was not deemed conclusive. New cohorts were set up earlier to evaluate 2 and 10 mg/kg Q3W in a randomized fashion and were starting to provide an answer.

An open question from earlier was answered unequivocally: was there non-linearity in exposure at low dose levels? The way to answer such a question is not to increase the N of already studied dose levels, such as 1 mg/kg, but to scan even lower doses to seek the point where clear target-mediated exposure patterns emerge. Rather uniquely, a cohort was added in which patients received step-down doses as far down as 0.005 mg/kg, even if for one week. Lo and behold, pharmacokinetics appeared linear, down to a

dose as low as 0.1 mg/kg, 10-fold lower compared to earlier signs (Patnaik et al., 2015).

An interdisciplinary group of scientists turned the study database upside down to evaluate potential causes for the initial finding of decreasing activity with decreasing dose. Among all explanations, the impact of baseline tumor size stood out, as was published later (Joseph et al., 2018). Pembrolizumab is effective across a wide range of tumor burden, but is clearly more efficacious at lower baseline burden. Uncontrolled, the inclusion of progressively sicker patients over time during early expansion resulted in a completely phantom dose-response. Thanks to strong sponsorship and openness throughout the company to new evaluation methods, such as QSP modeling, the conclusion was reached in time.

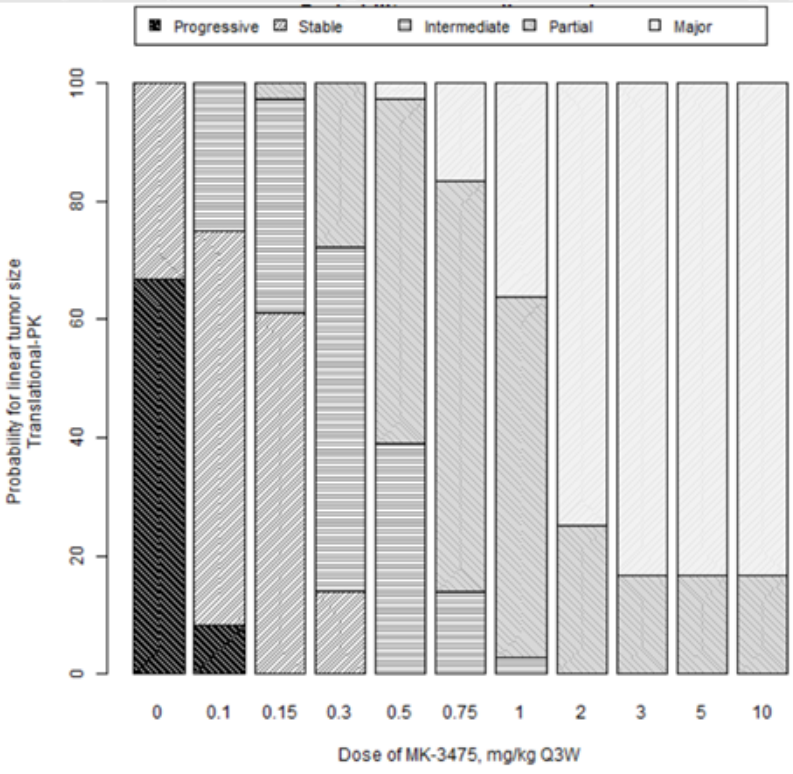
Dose selection was still not out of the woods, as it, in early 2013, could not be pinned down to what extent these findings were due to random chance. Also, the updated and more robust PD analysis coming out of the step-down doses was inconclusive, as it reflected the situation in blood rather than the tumor.

Mouse in the clinic

Far away from these evaluations and discussions, the animal pharmacology group in Palo Alto carried on. Final data were delivered that, for the first time, could make a quantitative connection between exposure, receptor occupancy in the tumor and tumor inhibition. Mice with intact immune system, syngeneic mouse models, in contrast to the ubiquitous xenograft mouse models, leveraging the 'mousified' version of pembrolizumab, were critical. Data reflected earlier observations on the expansion of the PD-1 receptor in tumors upon dosing, and QSP modeling was now able to track these intricacies over time and dose. The model was translated to the human-clinical situation across scenarios of different hypotheses around human tumor growth and pertinent methods to scale drug effect relative to it. The mouse-based volumetric predictions were rescaled to reflect RECIST-based one-dimensional tumor size, as is commonly used in the clinic (Elassaiss-Schaap, 2010). The overall conclusion was that a dose of about 2 mg/kg was the lowest to provide maximal efficacy. The impact of uncertainty in model parameters and related hypotheses was evaluated and provided insights into critical aspects of the model, biology, and clinical knowledge alike (Lindauer et al., 2017).

The new evaluation provided a robust scientific rationale for selecting 2 mg/kg Q3W as the recommended dose. The decision to select 2 mg/kg was clear to all involved, and from that point onward, the team pivoted to fully support this dose. The direction was clear, and it was full steam ahead to the milestone of regulatory approval. The BLA was submitted with the report of a single clinical study and five modeling reports, a ratio very different from routine filings. Approval was reached later in the same year (2014) ahead of the approval of nivolumab a couple of months later. The FDA published their ClinPharmSummary, with statements supported predominantly through modeling approaches. One of the published figures was that of the translational modeling, with a statement reflecting that the dose selection was confirmed on the basis of this effort, see Figure 5.

Figure 3: Estimated Pembrolizumab Dose-Response for Probability of Anti-Tumor Activity Using Translational PK/PD Indicates Near-Maximal (> 90% Probability of Partial and Major) Responses Starting at Dose Regimens of 1 or 2 mg/kg Q3W



Source: Summary of Clinical Pharmacology Studies, Figure 2.7.2:2, Page 18

Figure 5: *Dose-response properties of pembrolizumab as established through translational QSP modeling, published by the FDA in the Clinical Pharmacology Summary of the Keytruda file (FDA, 2014).*

Epilogue

The stories of pembrolizumab carry on, with new indications still being identified and new studies being started, with more than 2000 trials registered on clintrials.gov. Consistent with the observations on the impact of tumor burden, the drug is also approved for (neo-)adjuvant (early stage) diseases, for example. All kinds of details have been left out, and stories about different modeling aspects, clinical paradigms and regulatory interactions could also have been told.

Conclusion

The discovery and development of pembrolizumab is a story of peaks and shallow valleys, highlighting the need to embrace new paradigms together with robust science to bring products of a new mechanism to the market.

About PD-value and contact

PD-value is a modeling service provider with deep expertise in translational pharmacometrics, QSP, mathematical and systems modeling. PD-value is privately owned, based in the Netherlands and consists of a growing group of 15 experts to assist in bringing drug discovery and development programs forward by adding more value to client data. The author can be contacted by email at jeroen@pd-value.com or through www.linkedin.com/in/jeroenes.

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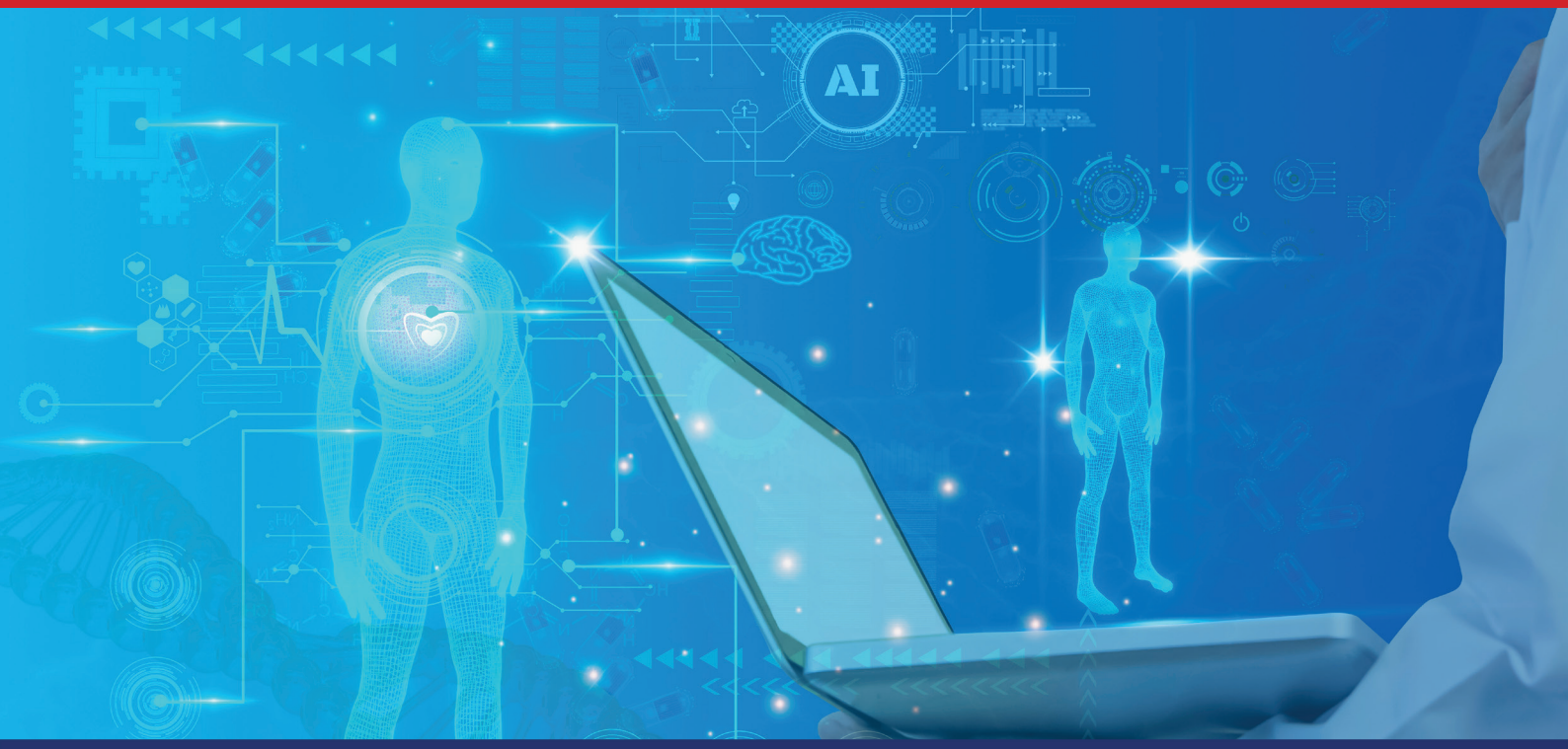

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SUMMARY OF A PUBLISHED STUDY USING CAF®09B

Sofia Popov, Scientific Writer

A new study1 published in Frontiers in Immunology reports promising vaccine results for patients with hormone-sensitive prostate cancer. The researchers demonstrated that a vaccine formulation containing the novel adjuvant CAF®09b and a Bcl-XL-peptide was able to elicit potent CD4+ and CD8+ T-cell responses. The team believes these findings can have an influential impact on the future of prostate cancer treatment. Dennis Christensen, Croda's Head of Global R&D, says that the «data shows that CAF®09b is a promising adjuvant for antigen-specific cancer immunotherapies».

Globally, prostate cancer (PC) ranks as the second most diagnosed cancer and the fifth primary cause of cancer-related mortality in men. Current treatment options for metastatic disease are limited, and the prognosis is poor where androgen deprivation therapies fail. Thus, new therapies are desperately needed.

Cancer immunotherapy

Tremendous progress has been made in the field of cancer immunotherapy, with investigations underway for their efficacy in a variety of cancer types. Therapeutic vaccines that amplify tumour-specific T-cell responses through immunisation are of particular interest. Tumour-specific and tumour-associated antigens (TAA) are found to be expressed in high numbers in PC. Moreover, PC patients often have T-cells specific for certain prostate-specific antigens in their peripheral blood, indicating the potential for therapeutic vaccines to support PC-specific T-cell immunity.

Although TAA can be found in healthy cells, they are generally elevated in cancer. Thus, they make useful targets and peptide-based vaccines capitalise on that: they typically consist of a TAA-derived amino acid sequence.

Peptide cancer vaccines require the presence of CD8+ and CD4+ epitopes to activate cytotoxic T-cells (CTLs) for effective anti-tumour immunity. Moreover, activation of T-helper cells is necessary to sustain the CTLs effector function.

Bcl-XL overexpression in prostate cancer

Researchers investigated Bcl-XL, an anti-apoptotic protein overexpressed in PC. Increased Bcl-XL expression has been associated with less responsiveness to chemotherapy and a poorer prognosis. Pre-clinical studies have demonstrated that cancer patients can have a naturally occurring T-cell response against epitopes derived from the Bcl-XL protein. These T-cells can even directly kill cancer cells that overexpress Bcl-XL. Research has demonstrated that suppressing Bcl-XL facilitates apoptosis, therefore sensitising the cancerous cells to chemotherapy and radiotherapy. Conversely, increased levels of Bcl-XL expression can lead to multi-drug resistance.

The Bcl-XL-peptide-CAF09b vaccination

For this vaccine formulation, the Bcl-XL_42 peptide was used, which contains 42 amino acids. Such longer peptides can induce more robust and diverse immune responses since they cannot directly bind to major histocompatibility complex I (MHC-I) and must be processed by antigen-presenting cells (APCs.) In turn, this helps stimulate both CD4+ and CD8+ T-cells.

The researchers administered the Bcl-XL_42 peptide into CAF09b, a novel liposomal vaccine adjuvant. CAF09b is comprised of lipid surfactants (DDA and MMG) and the TLR3 agonist poly(I:C).

Pre-clinical models have demonstrated CAF09b's superior capacity to shift the immune response towards a type I/CD8+ T-cell response. CAF09b increases the uptake of peptides by APCs, activating them to

stimulate cross-presentation and proinflammatory signalling that can therefore activate vaccine-specific CD4+ and CD8+ T-cells.

The study

For this investigation, the scientists looked at 20 adult patients with hormone-sensitive PC that were scheduled to begin hormone therapy. The team split the patients into two groups: Group A and B. Group A received three vaccines via intramuscular injection (IM,) and then intraperitoneal injection (IP.) Group B received the vaccines in reverse order. The vaccine-induced immune responses were then analysed using Enzyme-Linked Immunospot and flow cytometry.

Overall, the vaccine was shown to be safe and tolerable. The most common side effects were fatigue and mild pain at the injection site. Fatigue may also be explained by concomitant bicalutamide treatment – commonly used for metastatic prostate cancer.

ELISPOT analyses showed significant Bcl-XL_42 specific T-cell responses for both groups. Nevertheless, patients had stronger and earlier immune responses after IP injection than after IM injection, supporting suggestions that this administration route could lead to better antigen presentation leading to a CD8+ T-cell response. Furthermore, the researchers illustrated an increase in T-cell activation markers CD107a and CD137.

Closing remarks

Ultimately, these findings showed that the Bcl-XL-peptide-CAF09b vaccination is both safe and feasible for patients with hormone-sensitive PC. The vaccine carries the capacity to elicit CD4+ and CD8+ T-cell responses, with IP administration demonstrating greater potency.

1.Mørk and al., "First in Man Study", read the full article here:

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