JANUARY 2022 Volume 3, Issue 1

IMMUNO-ONCOLOGY INSIGHTS

SPOTLIGHT ON:

Anticipating immuno-oncology modality/platform development trends for 2022

IMMUNO-ONCOLOGY INSIGHTS

Volume 3, Issue 1

Anticipating immuno-oncology modality/platform development trends for 2022

EXPERT INSIGHT

A need to update paradigms for myeloid cells within the tumor microenvironment to advance immunotherapy

Michelle Kinder, PhD

There are controversies and conflicting hypotheses about myeloid cells within the tumor microenvironment. On one hand is the theory that macrophages need to be depleted; on the other, the idea that macrophages need to be re-programmed from M2 to M1 phenotype. There is a lot of discussion surrounding myeloid derived suppressor cells within the tumor microenvironment but the frequency, phenotype, and functional role of these cells are not well defined. Herein, we will review the current understanding of the myeloid cells within the human tumor microenvironment and their possible function with a focus on myeloid derived suppressor cells, granulocytes and macrophages. We will also explore different immunotherapeutic approaches to engage these immune cells within the tumor microenvironment. Furthermore, approaches on how we can characterize the role and function of myeloid cells within the solid tumors are described.

Immuno-Oncology Insights 2022; 3(1), 25–36

DOI: 10.18609/ioi.2022.004

Immunotherapy was first conceived using "Coley's toxin" which was a mix of heat-killed bacteria administered into patients. William Coley first hypothesized that using bacteria to treat cancer after a patient with a skin infection resulted in regression of his tumor. During his time no one could explain how Coley's toxin worked. We now know

that this bacterial mixture worked to invigorate the immune response to cancer through stimulation of Toll-Like Receptors (TLRs) expressed primarily on myeloid cells. Today a weakened form of Mycobacterium bovis in Bacillus Calmette–Guérin (BCG) is used to treat bladder cancer since the 1970s and can be considered a derivative of the treatment paradigm from Coley's time.

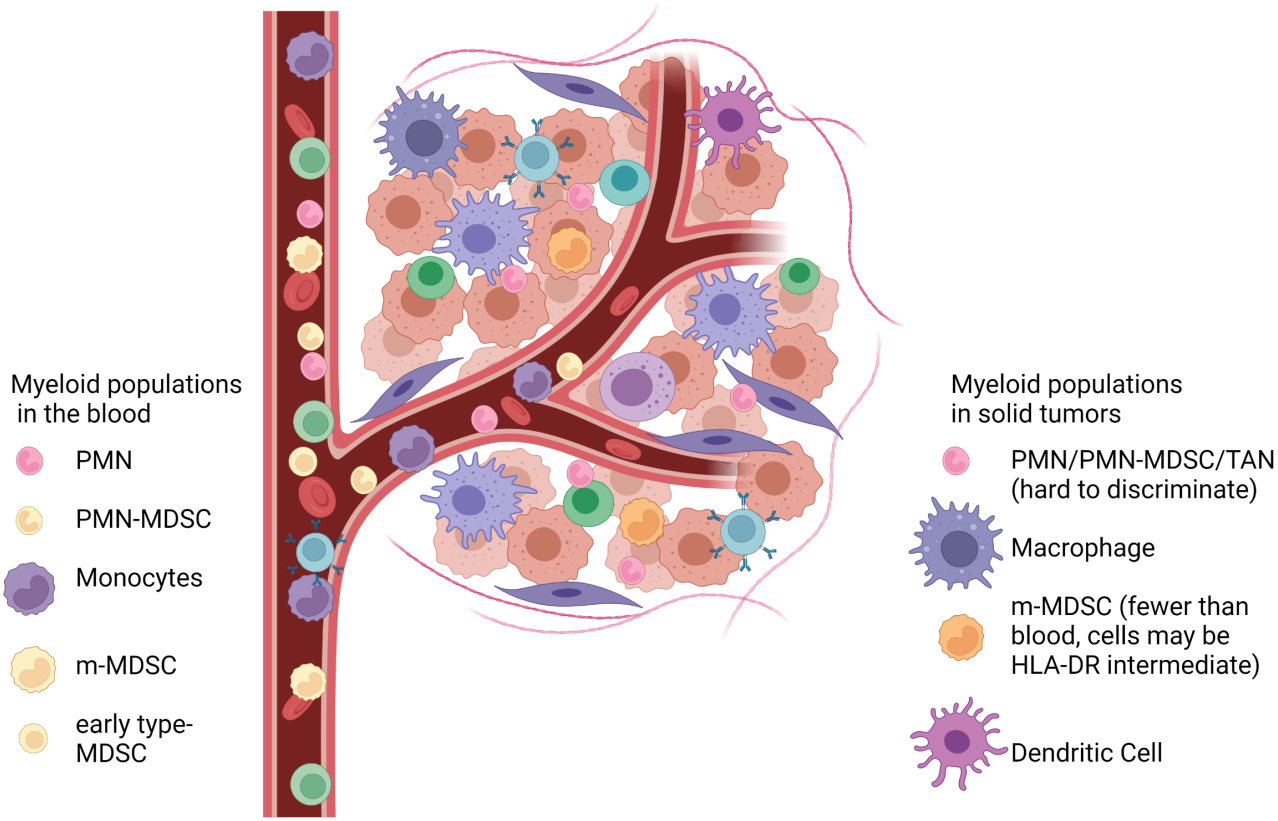
Targeting and stimulating myeloid cells within the tumor microenvironment has potential promise for immunotherapy due to these early observations. Myeloid cells compose a large portion of the tumor and include cell types such as macrophages, monocytes, myeloid-derived suppressive cells (MDSC), neutrophils, basophils, eosinophils, mast cells and dendritic cells. Many assumptions about the human tumor microenvironment were made from mouse models. Definitions and phenotypic characterization of cells within the tumor microenvironment differ between human and mice, and thus while learning from mice studies are helpful, one cannot directly translate mice data to human data. In this review article, we will focus on defining the myeloid cells within the human solid tumor microenvironment (Figure 1).

MYELOID-DERIVED SUPPRESSOR CELLS

Myeloid-derived suppressor cells are generally defined as immature myeloid cells with the ability to suppress T cell function. MDSCs increase in the blood during early pre-malignancy and in cancer [1]. MDSC

FIGURE 1

Myeloid cells within the human solid tumor microenvironment.



DOI: 10.18609/ioi.2022.004

from the blood of in premalignant and malignant cancers can suppress T cell function in *ex vivo* assays. Studies have suggested that the prevalence and frequency of MDSC are associated with poor prognosis in multiple tumor types [2,3].

There are three types of MDSCs including monocytic MDSCs (M-MDSC) which are defined as Lin-HLA-DR-/low, CD11b+CD33+CD14+CD15-, Polymorphonucler myeloid-derived suppressor cells (PMN-MDSCs) defined as Lin-HLA-DR-/low, CD11b+,CD33+CD14-CD15+, and early MDSCs which are Lin-HLA-DR-/low, CD11b+, CD33+CD14-CD15-[4].

Monocytic MDSCs are monocyte precursors/immature monocytes. Within peripheral blood mononuclear cells, they compose a relatively low compared to monocyte population. Nevertheless, there is correlation of M-MSDC in the blood with the development of cancer and with prognosis in some tumor types including prostate cancer and colorectal cancer among others [5,6]. M-MDSC inhibit T cell function through multiple mechanisms including arginase, nitric oxide, VEGF and PGE2. M-MDSC can also produce IL-10 and TGF-β [4,7].

Within the tumor, the number and differentiation state of M-MDSC is less defined. There is a challenge to discriminate between the various myeloid subsets and define their immuno-suppressive function within solid tumors. Because of the plasticity of both monocytes and M-MDSC, local environments promote differentiation and changes in in protein expression impacting function [8]. While monocytes rapidly differentiate into macrophages in the tumor, the fate of M-MDSC is less known Within the tumor the majority of CD11b+CD14+ cells generally display high HLA-DR+ expression [9,10] which would identify these cells as macrophages. There is also a percentage of cells CD11b+CD14+ cells that are with HLA-DR intermediate with monocyte morphology [8]. The percentages of M-MDSC as defined by Lin-HLA-DR-/low, CD11b+CD33+CD14+CD15 is low and outnumbered by macrophages and granulocytes [11].

Another population of MDSCs are early type. These cells express neither CD14 nor CD15. They are a very small population, and their function is not yet clear. They do not exhibit suppressive activity and are not correlated with disease patient outcome [12].

PMN-MDSCs are immature neutrophils. They have an increased suppressive activity on a per cell basis compared to M-MDSC [12]. They suppress T cells in *ex vivo* assays using reactive oxygen species, nitric oxide and arginase. Similarly with M-MSDC, the levels of PMN-MDSC in the blood are associated with poor patient outcome [4,12].

PMN–MDSC VS NEUTROPHILS

Neutrophils may also play a role in tumor progression. A higher neutrophil to lymphocyte ratio in the blood is a negative predictor of outcome across multiple cancer types and in response to immunotherapy [13]. Neutrophil counts are a general marker of inflammation in the body and numbers of neutrophils may be related to the levels of PMN-MDSC and/or intratumoral neutrophils [13,14].

What is the difference between PMN-MDSCs and neutrophils? There is no marker alone that can differentiate between the two, particularly in the tumor microenvironment. This leads to many investigators labelling neutrophils within the tumor as PMN-MDSCs without performing any *ex vivo* assays to understand their suppressive or immune-stimulatory function.

In the blood, when PBMCs are isolated using a Ficoll gradient, mature neutrophils are high density and will move through the gradient and be removed whereas PMN-MDSC are low density would be isolated with the PBMC layer [7]. Additional low density activated neutrophils may also be isolated in the PBMC layer [12]. It has been proposed that LOX1 may be a specific marker of PMN-MDSCs since it is increased on PMN-MDSC compared to neutrophils [15]. However, LOX1 is expressed on both neutrophils and

PMN-MSDC albeit at different levels limiting the utility of LOX1.

Because of the challenges defining neutrophils from PMN-MDSC in the blood, there is even more difficulty classifying these populations within a tumor where density gradients are not feasible to use. Perhaps, the more relevant question in this setting should be the function of these cells within the tumor. Functional studies are rarely done to define if these cells in the tumor are pro-tumorigenic or anti-tumorigenic. In fact, neutrophils isolated from tumors have immuno-stimulatory properties when cultured with human T cells *ex vitro* [16].

To add layer of complexity, studies have suggested that there are both pro-tumorigenic (N2) tumor associated neutrophil (TAN) and anti-tumorigenic (N1) TANs. N2 TANs are induced by TGF-β and are characterized by lower levels of ICAM-1, decreased formation of neutrophil extracellular traps (NETs) and decreased tumor cell-killing capacity [17]. N2 TANs can express IL-23, IL-6 and TGF-b which can potentially lead to Th17 skewing in the tumor microenvironment and thus a less robust anti-tumor T cell immune response [18]. Others have suggested the N2 designation is similar to PMN-MDSC cells [4]. N1 TANs are induced by type 1 IFNs and have increased ICAM-1 expression, increased formation of NETs and higher tumoricidal capacity. They express pro-inflammatory cytokines IL-12 and TNF-α [18]. Administration of high dose IFN therapy to melanoma patients, resulted in an increase of the N1 phenotype [19]. The use of N1 and N2 nomenclature is an over-simplification and likely TANs can be pro-tumorigenic or anti-tumorigenic based on levels of cytokines and activation states within the tumor microenvironment. There may not be a defined dichotomy of function but rather a functional plasticity based on context.

Tumors as well as other immune cells express IL-8 (CXCL8), a chemokine which recruits neutrophils via CXCR1/2. IL-8 also functions in neutrophil activation and contributes to angiogenesis and EMT. High baseline levels of IL-8 in the blood in consistently associated with poor prognosis across multiple tumor types and poor response to immunotherapy [20]. Multiple CXCR1/2 inhibitors are currently in clinical trials in multiple disease settings. Of interest is an anti-IL8 antibody, BMS-986253, being tested in combination with nivolumab. These trials will help to determine whether IL-8 and subsequently neutrophils are a marker or a driver of resistance to anti-PD1 therapies.

MACROPHAGES

Macrophages comprise a significant portion of the tumor microenvironment [11]. Monocytes and immature myeloid cells that are attracted to the tumor microenvironment likely rapidly differentiate to macrophages [8]. A variety of tumors express chemokines including M-CSF, GM-CSF MIP1α, MIP1β, and MCP-1 to attract and differentiate myeloid cells. Using flow cytometry, multiple groups have shown that CD11b+CD14+ cells have elevated HLA-DR within the tumor microenvironment suggesting their maturation [9,10].

The framework of M1 and M2 is difficult to apply to tumor associated macrophages. M1 macrophages are 'classically activated' macrophages that are pro-inflammatory. *In vitro*, they are differentiated with IFN-γ and LPS. They express high levels of IL-12 and low levels of IL-10. Markers for M1 macrophages have been reported to be CD64, CD80, and CD86 [21]. Meanwhile there are multiple different types of M2 macrophages such as M2a, M2b, M2c and M2d [22]. M2a macrophages are "alternatively activated' and differentiated *in vitro* with IL4 and IL-13. They express high levels of CD206. M2a cells are macrophages thought to mediate responses to wound healing. M2c cells likely more closely replicate macrophages from the tumor microenvironment. They are differentiated with IL-10 and TFG-β which are abundant in the tumor microenvironment. They are characterized by the expression of CD163 [22].

DOI: 10.18609/ioi.2022.004

Characteristics of TAMs are beyond what is developed *in vitro* with cytokine cocktails. Tumors co-express M-CSF, GM-CSF, MIP-1α, MIP1β, MCP-1, IL-10, TGF-β, IL-8, IL-6 etc. There are likely localized spatial gradients to the levels of cytokines and differences based on the tumor type and location.

TAMs within the microenvironment tend to have expression of BOTH M1 AND M2 receptors rather than a dichotomy of expression. TAMs can co-express CD206, CD163, CD80, and CD86 [9,10]. Therefore, the use of M1 and M2 within the tumor microenvironment is limiting as tumor associated macrophages can express properties of both.

Similarly with neutrophils, macrophages within the tumor microenvironment can simultaneously have anti-tumorigenic and pro-tumorigenic properties. TAMs express IDO, arginase, TGF-β and IL-10 which can limit T cell proliferation. On the other hand, activated macrophages have the ability to phagocytose tumor cells and contribute to expression of IL-12p40 and IFNs.

The overall depletion of macrophages by inhibition of CSF1R has not been fruitful thus far. There is no single agent activity except in tumors that are directly driven by the ligand M-CSF such as tenosynovial giant cell as seen with treatment with the CSFR1 inhibitor PLX3397 in patients [23]. However, PLX3397 is ineffective against recurrent glioblastoma [24], and a different CSF1R inhibitor, LY3022855, is ineffective against breast or prostate cancer [25]. Merely depletion of macrophages and immature monocytes does not result in effective immunotherapy.

Rather than deplete TAMs, another approach is to take advantage of their plasticity and activate them via receptors such as CD40 or TLRs. This approach activates macrophages in the tumor microenvironment and also re-invigorate a productive T cell response.

The role of anti-CD40 therapeutics in solid tumors is still under investigation. Administration of an anti-CD40, Selicrelumab, in a neoadjuvant pancreatic tumors results in macrophage activation resulting in decreased fibrosis, less M2 immune phenotype, dendritic cell maturation, and modulation of pro-inflammatory cytokines [26]. However, in a recent phase 2 randomized trial of anti-PD1(Nivolumab), Gemcitabine plus Nab-paclitaxel and a different anti-CD40 agonist, Sotigalimab, in previously untreated metastatic pancreatic cancer, the combination arm of Sotigalimab, Nivolumab and Gemcitabine, Nab-paclitaxel did worse than Nivolumab alone with Gemcitabine plus Nab-paclitaxel [27]. It is not clear why CD40 activation led to a decreased response rate in the combination. Thus far it is unclear how to proceed with CD40 activation as therapy for solid tumors. Timing and the number of doses may play a role in maximizing the anti-tumor activity from anti-CD40 therapeutics.

Another method of activating macrophages include the use of TLR agonists. There are currently three approved TLR agonists on the market including BCG for bladder cancer which is a TLR2/3 agonist. The other approved TLR agonists are the TLR4 ligand monophosphoryl lipid A for cervical cancer and the TLR7 agonist imiquimod for basal cell carcinoma [28]. TLRs do not just activate macrophages, they also activate dendritic cell types depending on the TLR expression. TLRs serve as adjuvant for vaccines and can bridge the adaptive and innate immune systems by increasing antigen presentation. Additional TLR agonists are in clinical trials for the treatment of various cancers.

Macrophages have a clear role in the tumor to mediate antibody phagocytosis and killing of tumors particularly in the context of therapeutic antibodies. The presence of macrophages is positively associated with outcome in the context of antibody mediated therapy [29,30]. It is likely that TAMs play an important role in mediating responses to therapeutic antibodies in tumor patients [9].

The variable regions of therapeutic antibodies bind to a tumor antigen while the constant portion of an antibody binds to Fc receptors expressed on immune cells. Tumor associated- macrophages express activating Fc receptors CD64 (FcγR1), and CD32a (FcγR2a) while a subset of TAMs also express

TABLE 1

Techniques for assessing the tumor microenvironment in human solid tumors.

Sample Type	Technique	Pros	Cons
FFPE block/ Frozen	RNAseq, targeted RNA seq	Relatively easy to obtain; exploratory analyses can be done	Difficult to determine which cells are present and which cells change their expression
	Immunohistochemistry	Straightforward; Can analyze whole sections	Limited to one marker of interest and serial sections
	Multiplex Immunofluorescence	Spatial information; examine co-expression; multiple markers analyzed	Limited number of markers at once; lower resolution due to nature of fluorescence
	Multiplex immunochemical consecutive staining	Spatial information; up to 14 markers per panel	Limited availability
	Imaging mass cytometry	Spatial information; Up to 40 analytes per slide with single cell resolution	Time consuming to acquire; Limited to validated metal tagged antibodies
	Spatial multiomics (10× genomics, akoya, nanostring, etc)	Spatial information+ RNA expression+/- protein expression	A lot of data; Challenging to analyze
Fresh dissociated cells	Flow cytometry	Singe cell resolution of cell populations; Up to ~18 protein markers per panel	Need for fresh tumors; Limited data analysis; Difficult to assess many intracellular proteins; Lack of spatial information
	Mass spectrometry (Cytof)	Up to 40 analytes per cell; Intracellular and extracellular proteins	Need for fresh tumors; Time consuming; Lower sensitivity for low expression; Lacks spatial information
	Single cell RNA sequencing	Characterization of individual cell types and gene expression	Need for fresh tumor cells; Lacks spatial information

CD16 (FcγRIII). Engagement of an activating FcγR with antibody opsonized tumor cells generally results in phagocytosis of the tumor cell resulting in cytokine release and tumor cell killing [9]. Macrophages can also mediate trogocytosis which is the removal of the engaged receptor from the surface of a tumor cell. For example, Amivantamab which is a bispecific EGFRxcMET antibody that is Fc engineered for enhanced Fc receptor binding targets tumor cells through phagocytosis and killing [31]. It also works in part by reducing levels of EGFR and cMET receptors resulting in decreased tumor cell proliferation [32]. Once can envision that antibody mediated therapy for solid tumors, works in part through arming and redirecting TAMs against tumors.

CHALLENGES & OPPORTUNITIES FOR CHARACTERIZING THE TUMOR MICROENVIRONMENT IN PATIENTS

There is a need to better characterize the human tumor microenvironment in patients using cutting age techniques to better understand the myeloid cells and improve therapeutics. There is a myriad of advanced technologies available to resolve immune cells within the tumor microenvironment on the single cell level [33] (Table 1).

The traditional approach of single-color immunohistochemistry is restrictive because multiple markers are needed to define cell types. Even using multi-color immunofluorescence it is still challenging to define and understand the cells within the tumor. For example, monocytic MDSC are defined in part by Lin-CD11b+HLA-DR-CD14+, 5+ markers are necessary to accurately define these cells [4].

DOI: 10.18609/ioi.2022.004

RNA sequencing has been widely used to examine tumor tissues. In contrast to many techniques, more exploratory analyses can be done since it is not limited by pre-determined markers of interest. However, it is difficult to deduce which cells are present. Immune signatures such as CIBERSORT are generated based on *in vitro* or *ex vivo* immune cells and may not reflect unique biology of the tumor microenvironment [34]. Furthermore, it is not possible to determine which cells are changing their gene expression.

Single cell approaches have been utilized to characterize immune cells but require the use of fresh tissue. Fresh tissue is challenging to collect and dissociate, in particular for metastatic lesions. Flow cytometry analysis provides information on the phenotypic characterization of the cells at the single cell level. It has been used to characterize the immune microenvironment of breast cancer [9,35] and NSCLC cancer [10,11] among others. Because of differences in dissociation protocols, antibody clones, panels, and gating analyses there are some differences between group to group. However, flow cytometry can reliably determine multiple protein markers on a single cell. Flow cytometry is limited by the need for pre-determined antibody panels and the number of antibodies per panel is relatively limited.

Mass cytometry or cytometry by time of flight (Cytof) can increase the number of markers used compared to flow cytometry - up to 40 analytes can be tested in a single panel. Mass cytometry can be limited by sensitivity for low expression markers. Mass cytometry is time-consuming for acquisition of the samples and the availability of metal-tagged antibodies are more limited compared to antibodies available for flow cytometry.

Single cell RNA sequencing greatly expands the number of analytes tested per cell with the ability of measuring whole transcriptomes or selected genes. Single cell RNA sequencing techniques have also been expanded such that there is the ability to include protein expression markers as well as RNA analysis. Using higher content analyses one can use unsupervised clustering such as t-distributed stochastic neighbor embedding (t-SNE) to visualize and understand populations in the tumor environment.

By leveraging both bulk sequencing and single cell RNA sequencing approaches, investigators have determined a myeloid cell resistance mechanism for anti-PD(L1) therapies in urothelial cancer [36]. Bulk RNA sequencing data was available from two different clinical trials using atezeoliumab or nivolumab in metastatic urothelial cancer that had outcome data associated with it. Investigators used additional single cell RNA sequencing data from urothelial cancer patients to resolve the RNA signatures associated with poor prognosis. This study demonstrated that there was heterogeneity of the myeloid populations that were beyond classical M1 and M2 polarization. In addition, poor response to anti-PD(L1) was associated with myeloid cells that had increased pro-inflammatory cytokines/chemokines (IL1B, CXCL8 (IL8), SPP1, and CCL20), decreased antigen presentation genes and were unrelated to M1/M2 polarization [36]. The use of combining bulk sequencing with further refinement from single cell sequencing could have additional implications for understanding responsiveness to therapeutics.

Single cell sequencing was also used to profile NSCLC and corroborated the analyses with CITE-seq which combines protein markers with transcriptional information [37]. Investigators demonstrated there was a large population of mononuclear phagocytic cells within NSCLC. Intratumoral dendritic cells were characterized by CD14+CD163+ DC3 cells. Tumors were found to be dominated by monocyte-derived macrophages that were distinct from alveolar macrophages. Within this cluster of monocyte-derived macrophages, four subtypes were determined. A particular cluster which expressed high levels of SPP1, glycolysis genes and lower levels of HLA Class II tracked with responders to anti-PD(L)1 therapy whereas populations of DC1, DC2, avelolar macrophages and Azu+ macrophages tracked with nonresponders

[37]. Single cell sequencing allows to understand the level of heterogeneity of myeloid cells well beyond what can be done with pre-determined panels of analytes.

The ability to obtain enough fresh tissue for single cell analyses is often not possible, especially in the case of analyzing metastatic lesions. In addition, because the tumor microenvironment has cytokine gradients that can influence cellular differentiation and gene expression, the location of each cell within the tumor may provide clues to its function. High content imaging allows for spatial characterization of immune infiltrates.

One example of enhanced spatial imaging has been a technique using a multiplexed IHC based on serial chromogenic staining (Multiplex Immunochemical Consecutive Staining) which allows for up to 14 stains to be analyzed on a single slide. This information was used to characterize the immune contexture of pancreatic cancer [38] as well as characterize the impact of CD40 in a window of opportunity study in pancreatic cancer [26].

Another example of enhanced spatial imaging is imaging mass spectrometry such as that offered by Fluidigm. This technique combines the power of mass cytometry with spatial awareness. It still has the limitations of mass imaging but can have up to 40 protein analytes on single cell resolution on a frozen or FFPE slide [39]. This technique was used on samples from the Keynote 177 and elucidated that antigen presenting macrophages (CD68+CD74+) interacting with CD8 T cells were associated with response to anti-PD1 [40].

Combined spatial imaging with large numbers of proteins or transcriptomics have become more accessible with vendors such as 10x Genomics, Nanostring and Akoya offering these services. High content spatial analyses will likely lead to additional advancements in the knowledge of myeloid cells within the tumor microenvironment [41]. Cross-referencing data across different technology platforms are needed to fully characterize and validate findings. Hopefully, enhanced characterization of the microenvironment, particularly in response to immunotherapy, will enable the design of the next generation of therapies and drug combinations.

HUMAN VS MOUSE MICROENVIRONMENT

Many of the hypotheses for human tumor microenvironment were developed in mice. However, there are significant differences in hematopoiesis in humans and mice. Mice are very quick to have extra-medullary hematopoiesis in the spleen when under stress which is why a significant portion of immature myeloid cells are readily found in spleens of tumor-bearing mice. Development of these immature myeloid cells in the spleen in mice are clearly associated with negative outcomes. Removal of the spleens to reduce the extra-medullary myelopoiesis results in reduced tumor burden in mice [42]. However, the role of extra-medullary hematopoiesis in humans is less clear. One study has shown increased levels of splenic CD11b+ and CD133+ (a marker for human stem cell progenitors) cells by IHC in multiple tumor types [43] but the level and contribution of splenic hematopoiesis in humans is not clear.

One major difference in tumor associated macrophages between mice and humans are the levels of expression of HLA-DR (MHC class II). Within the murine tumor microenvironment, a large portion of monocytic cells are HLA-DR negative [44] whereas, in humans the monocytic cells highly express HLA-DR [9,10]. Therefore, mice have a higher portion of immature myeloid cells in the tumor while humans have more mature myeloid phenotype. These differences can lead to discrepancies between mechanisms of action of therapeutics in mice versus humans. Additional models need to be developed to bridge the understanding of myeloid cells in mice versus humans.

There are also many other considerations for differences in the immune systems between mouse models and human tumors including aging of the immune system in older

DOI: 10.18609/ioi.2022.004

patients, the course and context of development of the tumor, differences in immune receptors [16,45].

TRANSLATION INSIGHT

When examining the tumor microenvironment, it is paramount to use high content multi-dimensional analyses for human tumors No single marker is sufficient for characterizing the immune environment. Combination with spatial imaging also may give clues to immune function and localized cytokine environments and gradients may influence immune function and phenotype.

Scientists need to create an understanding of the human tumor microenvironment independent from hypotheses generated in mice due to inherent differences in the biology of the immune system and limitations of tumor models. When examining human tumors, it is important to keep an open mind rather than infer based on mouse biology. Building better models of human tumor microenvironment such as using fresh human tumors *ex vivo* will be needed to test therapeutics rather than sole reliance on mouse models to enable more effective treatments in patients.

Moreover, it is important to recognize that immune cells in the blood are different in the tumor microenvironment. Within the tumor microenvironment there are different cytokine and environmental signals present that alter the function and phenotype of immune cells. Immune cells in the blood may change their differentiation, activation, phenotypic and functional states within a tumor. Analyses that examine the function of the immune cells within solid tumor are needed rather than extrapolating from blood assays.

The nature of both granulocytes and macrophages in the tumor microenvironment is complicated. Both can have pro-tumorigenic and anti-tumorigenic properties at the same time. Using a M1/M2 or N1/N2 framework is limiting and does not accurately describe the pleotropic nature of these cells. We tend to want to label cells as good (M1, N1) or bad (M2, N2) but in reality, macrophages and neutrophils can exhibit both pro-tumorigenic and anti-tumorigenic properties concurrently. Regardless of labels increased pro-inflammatory cytokines such as type I IFNs (IFNa, IFNb), IFN-l and IL-12 can tip the scales for a more anti-tumor effect.

It is likely that both the engagement of innate and adaptive arms need to be utilized for the full impact of immunotherapy to be realized. TLR agonism may be successful in some tumor types because it results in a burst of antigen presentation leading to robust T cell responses. Inhibiting or even activating myeloid cells alone may not result in anti-tumor activity- Engagement of both innate and adaptive immunity may be needed for successful immunotherapy in solid tumors.

REFERENCES

1. Ma P, Beatty PL, McKolanis J, Brand R, Schoen RE, and Finn OJ. Circulating Myeloid Derived Suppressor Cells (MDSC) That Accumulate in Premalignancy Share Phenotypic and Functional Characteristics With MDSC in Cancer. *Front. Immunol.* 2019; 10, 1401.
2. Koh, J, Kim Y, Lee YK *et al.* MDSC subtypes and CD39 expression on CD8(+) T cells predict the efficacy of anti-PD-1 immunotherapy in patients with advanced NSCLC. *Eur. J. Immunol.* 2020; 50, 1810–1819.
3. Zhang S, Ma X, Zhu C *et al.* The Role of Myeloid-Derived Suppressor Cells in Patients with Solid Tumors: A Meta-Analysis. *PLoS. One.* 2016; 11, e0164514.
4. Bronte V, Brandau S, Chen S-H *et al.* Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat. Commun.* 2016; 7, 12150.
5. Idorn M, Køllgaard T, Kongsted P, Sengeløv L, Straten P. Correlation between frequencies of blood monocytic myeloid-derived suppressor cells, regulatory T cells and negative prognostic markers in patients with castration-resistant metastatic prostate cancer. *Cancer Immunol. Immunother.* 2014; 63, 1177–87.
6. Tada K, Kitano S, Shoji H *et al.* Pretreatment Immune Status Correlates with Progression-Free Survival in Chemotherapy-Treated Metastatic Colorectal Cancer

Patients. *Cancer Immunol. Res.* 2016; 4, 592–9.

7. Gabrilovich DI. Myeloid-Derived Suppressor Cells. *Cancer Immunol. Res.* 2017; 5, 3–8.

8. Bergenfelz C, K Leandersson. The Generation and Identity of Human Myeloid-Derived Suppressor Cells. *Front. Oncol.* 2020; 10, 109.

9. Grugan KD, McCabe FL, Kinder M *et al.* Tumor-associated macrophages promote invasion while retaining Fc-dependent anti-tumor function. *J. Immunol.* 2012; 189, 5457–66.

10. Singhal S, Stadanlick J, Annunziata MJ *et al.* Human tumor-associated monocytes/macrophages and their regulation of T cell responses in early-stage lung cancer. *Sci. Transl. Med.* 2019; 11.

11. Kinder M, Khaja Syed MM, Parker M *et al.* Evaluating CD38 as a Therapeutic Target in Non-Small Cell Lung Cancer (NSCLC). *J. Soc.Immunother. Cancer* 2017; P376.

12. Lang S, Bruderek K, Kaspar C *et al.* Clinical Relevance and Suppressive Capacity of Human Myeloid-Derived Suppressor Cell Subsets. *Clin. Cancer Res.* 2018; 24, 4834–4844.

13. Sacdalan DB, Lucero JA, and D.L. Sacdalan DL. Prognostic utility of baseline neutrophil-to-lymphocyte ratio in patients receiving immune checkpoint inhibitors: a review and meta-analysis. *Onco. Targets Ther.* 2018; 11, 955–1265.

14. Moses K, Brandau S. Human neutrophils: Their role in cancer and relation to myeloid-derived suppressor cells. *Semin. Immunol.* 2016; 28, 187–96.

15. Condamine T, Dominguez GA, Youn JE *et al.* Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci. Immunol.* 2016; 1, aaf8943.

16. Eruslanov EB, Singhal S, Albelda SM. Mouse versus Human Neutrophils in Cancer: A Major Knowledge Gap. *Trends Cancer* 2017; 3, 149–160.

17. Fridlender ZG, Sun J, Kim S *et al.* Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* 2009; 16, 183–94.

18. Shaul ME, and Fridlender ZG, Neutrophils as active regulators of the immune system in the tumor microenvironment. *J. Leukoc. Biol.* 2017; 102, 343–349.

19. Andzinski L, Kasnitz N, Stahnke S *et al.* Type I IFNs induce anti-tumor polarization of tumor associated neutrophils in mice and human. *Int. J. Cancer* 2016; 138, 1982–93.

20. Gonzalez-Aparicio M, Alfaro C, Significance of the IL-8 pathway for immunotherapy. *Hum. Vaccin. Immunother.* 2020; 16, 2312–2317.

21. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat. Rev. Immunol.* 2012; 12, 253–68.

22. Wang LX, Zhang S-X, Wu H-J, Rong X-L, Guo J. M2b macrophage polarization and its roles in diseases. *J. Leukoc .Biol.* 2019; 106, 345–358.

23. Tap WD, Wainberg ZA, Anthony SP *et al.* Structure-Guided Blockade of CSF1R Kinase in Tenosynovial Giant-Cell Tumor. *N. Engl. J. Med.* 2015; 373, 428–37.

24. Butowski N, Colman H, De Groot JF *et al.* Orally administered colony stimulating factor 1 receptor inhibitor PLX3397 in recurrent glioblastoma: an Ivy Foundation Early Phase Clinical Trials Consortium phase II study. *Neuro Oncol.* 2016; 18, 557–64.

25. Autio KA, Klebanoff CA, Schaer D *et al.* Immunomodulatory Activity of a Colony-stimulating Factor-1 Receptor Inhibitor in Patients with Advanced Refractory Breast or Prostate Cancer: A Phase I Study. *Clin. Cancer Res.* 2020; 26, 5609–5620.

26. Byrne KT, Betts CB, Mick R *et al.* Neoadjuvant Selicrelumab, an Agonist CD40 Antibody, Induces Changes in the Tumor Microenvironment in Patients with Resectable Pancreatic Cancer. *Clin. Cancer Res.* 2021; 27, 4574–4586.

27. O'Hara MH, O'Reilly EM, Wolff RA *et al.* Vonderheide, Gemcitabine (Gem) and nab-paclitaxel (NP) ± nivolumab (nivo) ± CD40 agonistic monoclonal antibody APX005M (sotigalimab), in patients (Pts) with untreated metastatic pancreatic adenocarcinoma (mPDAC): Phase (Ph) 2 final results. *J. Clin. Oncol.* 2021; 39, 4019.

28. Urban-Wojciuk Z, Mhan MM, Oyley BO et al, The Role of TLRs in Anti-cancer Immunity and Tumor Rejection. *Front. Immunol.* 2019; 10, 2388.

29. Taskinen M, Karjalainen-Lindsberg M-J, Nyman H, Eerola L-M, Leppä S. A high tumor-associated macrophage content predicts favorable outcome in follicular lymphoma patients treated with rituximab and cyclophosphamide-doxorubicin-vincristine-prednisone. *Clin. Cancer Res.* 2007; 13, 5784–9.

30. Canioni, D., *et al.* High numbers of tumor-associated macrophages have an adverse prognostic value that can be circumvented by rituximab in patients with follicular lymphoma enrolled onto the GELA-GOELAMS FL-2000 trial. *J. Clin. Oncol.* 2008; 26, 440–6.

31. Grugan KD, Dorn K, Jarantow SW *et al.* Fc-mediated activity of EGFR x c-Met bispecific antibody JNJ-61186372 enhanced killing of lung cancer cells. *MAbs.* 2017; 9, 114–126.

DOI: 10.18609/ioi.2022.004

32. Vijayaraghavan S, Lipfert L, Chevalier K *et al.* Amivantamab (JNJ-61186372), an Fc Enhanced EGFR/cMet Bispecific Antibody, Induces Receptor Downmodulation and Antitumor Activity by Monocyte/Macrophage Trogocytosis. *Mol. Cancer Ther.* 2020; 19, 2044–2056.

33. Davis-Marcisak EF, Deshpande A, Stein-O'Brien GL *et al.* From bench to bedside: Single-cell analysis for cancer immunotherapy. *Cancer Cell* 2021; 39, 1062–1080.

34. Newman AM, Liu CL Green MR *et al.* Robust enumeration of cell subsets from tissue expression profiles. *Nat. Methods* 2015; 12, 453–7.

35. Ruffell B, Au A, Rugo HS Esserema LJ, Hwang S, Coussens LM. Leukocyte composition of human breast cancer. *Proc. Natl Acad. Sci. USA.* 2012; 109, 2796–801.

36. Wang L, Sfakianos JP, Beaumont KG *et al.* Myeloid Cell-associated Resistance to PD-1/PD-L1 Blockade in Urothelial Cancer Revealed Through Bulk and Single-cell RNA Sequencing. *Clin. Cancer Res.* 2021; 27, 4287–4300.

37. Leader AM, Grout JA, Maier BB *et al.* Single-cell analysis of human non-small cell lung cancer lesions refines tumor classification and patient stratification. *Cancer Cell* 2021; 39, 1594–1609 e12.

38. Liudahl SM, Betts CB, Sivagnonam S *et al.* Leukocyte Heterogeneity in Pancreatic Ductal Adenocarcinoma: Phenotypic and Spatial Features Associated with Clinical Outcome. *Cancer. Discov.* 2021; 11, 2014–2031.

39. Elaldi, R., *et al.* High Dimensional Imaging Mass Cytometry Panel to Visualize the Tumor Immune Microenvironment Contexture. *Front. Immunol.* 2021; 12, 666233.

40. Bortolomeazzi M, Keddar MR, Montorsi L *et al.* Immunogenomics of Colorectal Cancer Response to Checkpoint Blockade: Analysis of the KEYNOTE 177 Trial and Validation Cohorts. *Gastroenterology* 2021; 161, 1179–1193.

41. Bassiouni R, Gibbs LD, Craig DW, Carpten JD, McEachron TA. Applicability of spatial transcriptional profiling to cancer research. *Mol. Cell* 2021; 81, 1631–1639.

42. Levy L, Mishalian I, Bayuch R, Zolotarov, Michaeli J, Fridlender ZG. Splenectomy inhibits non-small cell lung cancer growth by modulating anti-tumor adaptive and innate immune response. *Oncoimmunology* 2015; 4, e998469.

43. Wu C, Ning H, Liu M *et al.* Spleen mediates a distinct hematopoietic progenitor response supporting tumor-promoting myelopoiesis. *J. Clin. Invest.* 2018; 128, 3425–3438.

44. Wiehagen K, Girgis NM, Yamada DH *et al.* Combination of CD40 Agonism and CSF-1R Blockade Reconditions Tumor-Associated Macrophages and Drives Potent Antitumor Immunity. *Cancer Immunol. Res.* 2017; 5, 1109–1121.

45. Mestas J, Hughes CW. Of mice and not men: differences between mouse and human immunology. *J. Immunol.* 2004; 172, 2731–8.

46. Wu C, Ning H, Liu M *et al.* Spleen mediates a distinct hematopoietic progenitor response supporting tumor-promoting myelopoiesis. J. Clin. Invest. 2018; 128, 3425–3438.

47. Wiehagen K, Girgis NM, Yamada DH *et al.* Combination of CD40 Agonism and CSF-1R Blockade Reconditions Tumor-Associated Macrophages and Drives Potent Antitumor Immunity. Cancer Immunol. Res. 2017; 5, 1109–1121.

48. Mestas J, Hughes CW. Of mice and not men: differences between mouse and human immunology. J. Immunol. 2004; 172, 2731–8.

AFFILIATIONS

Michelle Kinder, PhD
Scientific Director, Incyte

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author is a current employee of Incyte, and a former employee of Janssen/Johnoson and Johnson. The author declares that they have no other conflicts of interest.*

Funding declaration: *The author received support from Incyte for meeting and travel costs. The author also owns Incyte stock options and Johnson and Johnson stock. The author received no other financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *10 Dec 2021;* ***Publication date:*** *date.*

DOI: 10.18609/ioi.2022.004

INTERVIEW

Leveraging LAG-3 for improved patient outcomes

PAUL BASCIANO is an executive director, development program lead at Bristol Myers Squibb, which he joined in 2015. While there, he has had various roles in oncology clinical drug development spanning from first in human through registrational studies, as well as leading the oncology clinical collaborations portfolio. Before joining BMS, Paul completed medical school at Columbia University College of Physicians and Surgeons, followed by a residency in internal medicine and fellowship in hematology and oncology at New York Presbyterian Hospital-Weill Cornell. While at Cornell, he held the position of Assistant Professor of Medicine as a clinician-scientist in hematology and oncology, treating patients with hematologic disorders and leading a translational research laboratory studying the role of the cytoskeleton in hematologic disorders.

Immuno-Oncology Insights 2022; 3(1), 7–11

DOI: 10.18609/ioi.2022.002

Q What are you working on right now?

PB: Right now, I am leading our relatlimab development team at Bristol-Myers Squibb. We are working in the hope that we can bring the potential of LAG-3 targeted therapies to appropriate patients.

Q Can you firstly frame of us the rationale behind pursuing LAG-3 checkpoint inhibition in the immuno-oncology context, including in the combination therapy setting?

PB: Lymphocyte activation gene (LAG)-3 is a cell surface molecule expressed on immune cells, including T cells. It can negatively regulate proliferation and effector T cell function. It was observed that LAG-3 has been upregulated in many tumor types.

Existing established immunotherapies such as PD-1 and CTLA-4 inhibitors have really transformed patient care across multiple tumors, both as monotherapies and in combination. However, we know that there are multiple other immune checkpoints identified, such as LAG-3, that allow the possibility of novel combinations of new checkpoint inhibitors to be explored, potentially improving outcomes for patients and optimizing benefit-risk profiles of treatments. This is where we think LAG-3 can come into play.

Q Can you expand on key recent advances in our biological understanding in this area - for instance, in terms of mechanism of action?

PB: There have been many advances recently. We and others have reported preliminary evidence of broad activity of LAG-3 targeting therapies in multiple tumor settings. This activity has been seen in tumors that typically respond to immuno-oncology (I-O) treatments, as well as in tumor types that are generally considered insensitive to I-O therapies.

We have even seen activity where checkpoint inhibitors have failed, such as in patients that have previously been treated with I-O therapies and had disease progression. The broad range of activity has been observed through various mechanisms for targeting LAG-3. There are blocking antibodies and there is also soluble LAG-3 itself that is administered therapeutically. However, there is still a lot to learn about how this pathway can be targeted through various mechanisms, and across various disease settings.

Q Can you highlight the most pressing remaining shortfalls in our understanding in this area?

"Lymphocyte activation gene (LAG)-3 is a cell surface molecule expressed on immune cells, including T cells."

PB: As with many cancer therapeutics, there is a major need to identify patients who are most likely to benefit from particular therapies through the use of selection markers. They can be either clinical selection markers or translational in nature. Selection markers can help us to identify the most appropriate patients to treat

DOI: 10.18609/ioi.2022.002

with a particular drug that targets a mechanism of action (MOA) or a combination of them.

We have had much progress in terms of understanding so far, but there is still a lot more to be learned. We are actively working to understand what selection strategies can be applied to LAG-3 combinations, whether those are already established ones in the field of immuno-oncology or novel ones more tailored to LAG-3 itself.

> "...there is a major need to identify patients who are most likely to benefit from particular therapies through the use of selection markers."

Q Are there any other particular challenges to address in developing a LAG-3 checkpoint inhibitor?

PB: One unique aspect is a general lack of monotherapy activity across the field of LAG-3 blocking antibodies. This is a real challenge because in oncology, we often look to where there is monotherapy activity of a drug to guide its future development. With LAG-3, that does not appear to be the case. This was identified early, and so our developmental activities have taken that challenge into account.

Even more challenging, when thinking about combinations with established I-O therapies, we naturally want to look where those I-O therapies are active, and we have seen promising activity using various modalities that target LAG3 in these spaces. However, we have now seen activity in places where I-O therapies do not have activity such as in the patients who progressed after prior I-O treatment, as well as in areas where I-O therapies have not shown activity to date. This presents real challenges in terms of prioritizing development, but there are also opportunities. We can go very broadly and potentially bring more effective therapies in areas we may not have otherwise considered previously.

Q Tell us more about Bristol-Myers Squibb's R&D work in the space to date, specifically - what studies have been/are being conducted?

PB: We have and are continuing to actively explore LAG-3 combinations across a broad range of tumor types, including melanoma, non-small cell lung cancer, hepatocellular carcinoma, colorectal cancer, and others. We are also further exploring novel combinations of LAG-3, including with other established and emerging I-O checkpoint inhibitors, and also with non-I-O mechanisms.

Q Looking further ahead, what's your vision for the future impact of LAG-3 checkpoint inhibition, both within and potentially beyond I-O?

"With the overall rapid pace of advancement in I-O therapy ... you can really start to imagine a future where patients can receive rationally selected regimens based on their unique clinical characteristics and/or tumor biology."

PB: The future is really wide open for LAG-3 checkpoint inhibition. As I mentioned, we have seen promising results in a spectrum of tumors and difficult-to-treat settings with LAG-3 combinations.

With the overall rapid pace of advancement in I-O therapy, both in terms of LAG-3 and other MOAs, you can really start to imagine a future where patients can receive rationally selected regimens based on their unique clinical characteristics and/or tumor biology.

You can envision these regimens potentially including I-O and non-I-O combinations in particular tumor settings. Overall, we are only at the beginning of the story in immuno-oncology. Now that we have seen such promising activity with novel MOAs such as LAG-3, we are understanding that it will have an important role to play in the next chapter.

Q Finally, could you sum up some key goals & priorities for your work over the next 12-24 months?

PB: To put it simply, my goal is to ensure that we learn as much as possible about what we can achieve with this newly validated I-O target, so that we can fully realize its potential for as many patients as possible and hopefully improve their lives.

AFFILIATION

Paul Basciano
Executive Director,
Bristol Myers Squibb

DOI: 10.18609/ioi.2022.002

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Basciano is a Bristol Myers Squibb employee and stockholder.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *Dec 7 2021;* ***Publication date:*** *Jan 25 2022.*

INNOVATOR INSIGHT

Advancing engineered cell & gene therapy with precision gene editing

John Lambourne

The increased range and accessibility of gene modification technologies has had a great impact on the cell and gene therapy field, with CRISPR-Cas9 in particular garnering increased attention in the last decade. But is it always the best tool for the job? In this article, CRISPR and base editing techniques will be compared – demonstrating the key advantages base editing technology can offer to the field in terms of safety and efficiency, and how increasingly precise gene editing approaches can help to deliver the next generation of advanced therapies.

Immuno-Oncology Insights 2022; 3(1), 37–49

DOI: 10.18609/ioi.2022.005

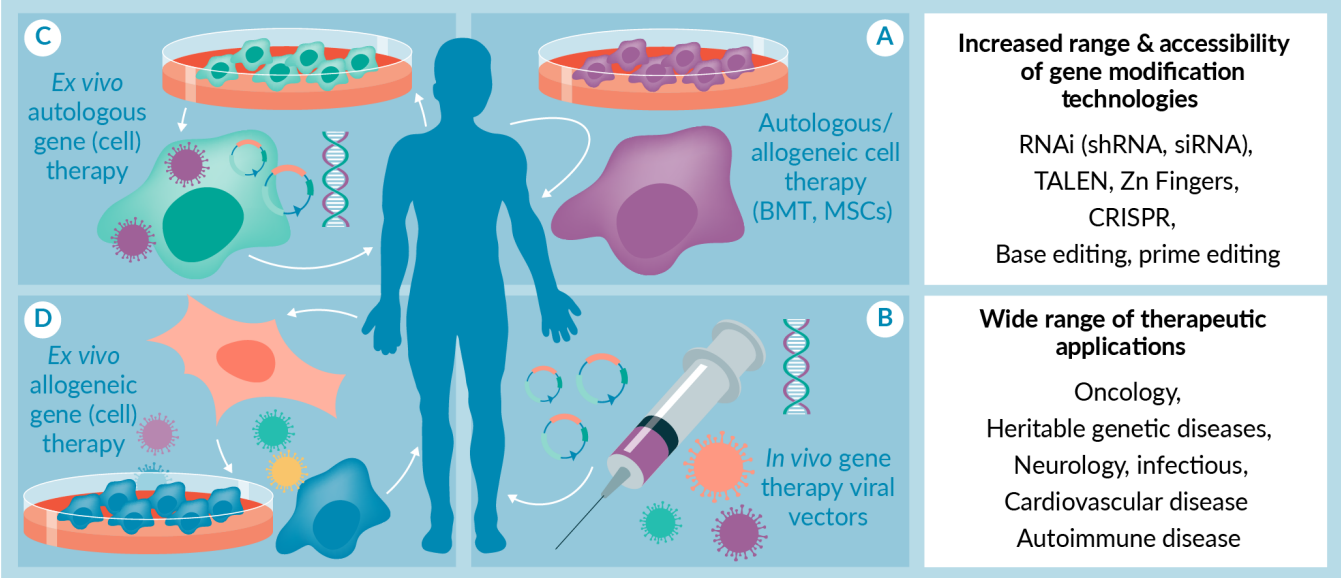
GENE MODIFICATION TECHNIQUES AT THE CENTER OF CELL & GENE THERAPIES

There are a number of gene modification technologies now available in the cell and gene therapy space, with a wide range of therapeutic applications. As seen in Figure 1, there are three key areas currently seeing growth – *ex vivo* autologous cell therapy, *ex vivo* allogeneic cell therapy, and *in vivo* gene therapy.

Gene modification technologies have been at the center of new therapies, and their evolution is enabling the development of novel therapeutics that are more precise, safer, and broader in application. Looking to the successes of the immuno-oncology field in particular, advanced therapeutic approaches are

FIGURE 1

Range and application of gene modification technologies.



proving successful in treating many unmet medical needs.

Engineered immune cell therapies have revolutionized the field of cancer therapeutics and are paving the way towards more personalized medicine. CAR-engineered T cells have proven to be particularly impactful for the treatment of hematological malignancies and are used not only to manage symptoms or delay relapse, but to cure disease. Examples include pioneers like Yescarta and Kymriah, and now include further therapies such as Tecartus, Breyanzi and Abecma.

However, these engineered immune cell therapies are not yet perfect, and gaps remain to be addressed, including:

- Side effects (cytokine release syndrome, neurotoxicity)
- Long-term efficacy
- Treatment accessibility (especially for autologous therapy)
- Deployment (cost, manufacturing logistics)
- Targeting solid tumors (delivery, immunosuppressive environment, variable antigens)

A dearth of safe target antigens has also hampered the development of CAR-T cells for broader therapeutic indications beyond CD19^{+}, leukemias, and lymphomas. Achieving durable response against solid tumors has been met by the challenges of poor T cell function in the immunosuppressive tumor microenvironment (TME) and the heterogeneity of antigen expression. High cost and poor scalability limit widespread access to these potentially life-saving treatments.

However, gene editing technologies have become increasingly accessible and have seen expanded application in cell and gene therapeutics. This will lead to a next generation of cellular products that harbor creative genetic manipulations to improve T cell potency and safety, and mitigate immunosuppressive triggers in the TME. Genetic engineering approaches could also enable the use of allogeneic cell sources, thereby improving scalability and allowing more complex fine-tuning and wider deployment of therapies.

BREAKING AWAY FROM THE DOUBLE STRAND BREAK

CRISPR-Cas9 has seen a great increase in popularity in the last decade. However, in

DOI: 10.18609/ioi.2022.005

some ways it is a relatively blunt tool – the double strand DNA breaks it creates can lead to a number of unintended consequences, including an uncontrolled indel profile, chromosomal rearrangements, translocations, and chromothripsis. As a result of these challenges, there has been a concerted effort to move to next-generation tools, such as base editing.

Base editing does not utilize double strand break technology, but instead relies on enzymatic activity. This provides a number of benefits, including an improved safety profile and high editing efficacy in non-dividing cells. As CRISPR works by indel formation, the results may be unpredictable. Base editing is more precise – coming in like a scalpel and removing just a few bases in a way that is predictable and provides the same outcome every time.

Some of the major advantages relate to base editing techniques being highly multiplexable. Multiple genes can be targeted without creating undesirable events in the cell, such as creating a high number of breaks or causing cell stress.

Therefore, although CRISPR and base editing are broadly similar technologies, these subtle next-generation changes make all the difference in the way they can be utilized.

EDITING WITH PINPOINT PRECISION

Horizon offers a specific base editing technology: Pin-point™ base editing (Figure 2).

Our approach to deaminase recruitment shortens the proteins in order to make them far more amenable to packaging and offers a highly tunable locus-specific control. This multifunctional approach allows you to have different loci with different things coming towards them. For example, aptamer A brings forward enzyme A, aptamer B brings forward enzyme B, and so on. This provides an element of control and optimization that other technologies do not have available.

BASE EDITING: CASE STUDIES

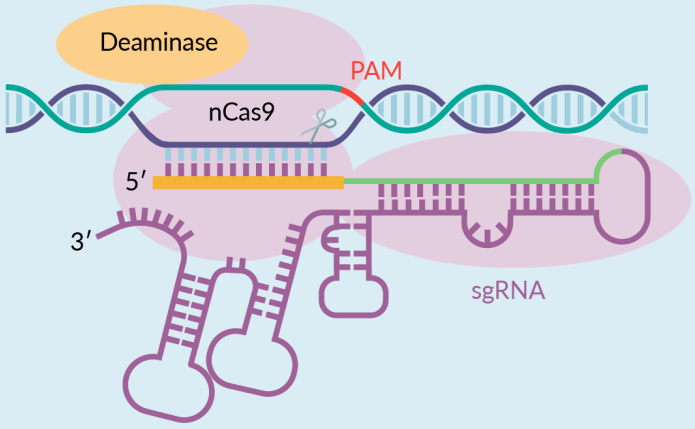
A number of tests were carried out to evaluate Horizon's base editing technology for various applications.

T cell engineering

At Horizon, we have directed a lot of focus to T cell engineering and multiplex gene editing, as in the clinic we are seeing a number of therapies requiring highly multiplex

FIGURE 2

Benefits of base editing as a gene editing tool.

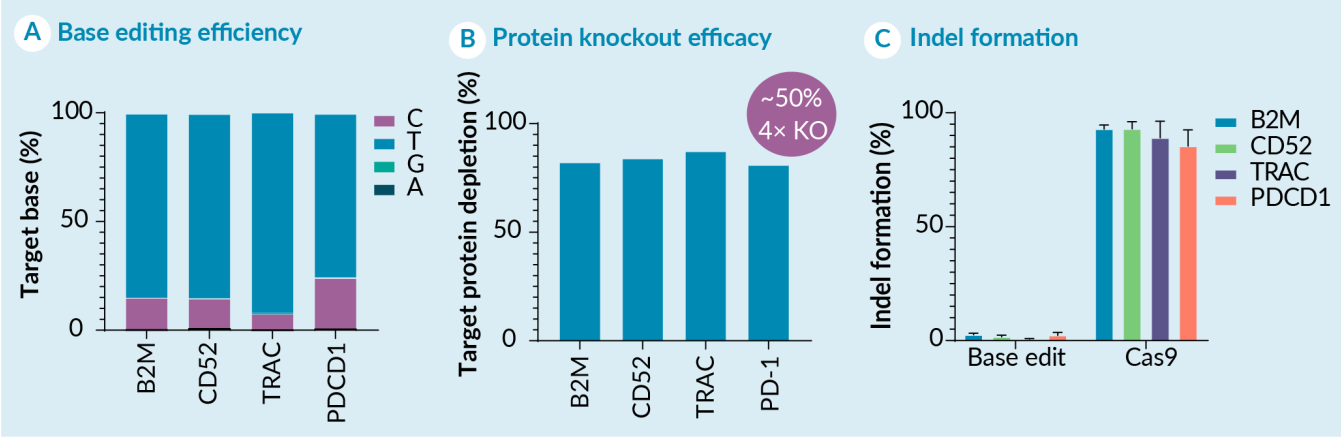


Benefits of base editing
- Predictable, precise and efficient editing outcome
  - Single- and multi-gene context
- No DNA double-strand breaks
  - Improved safety profile
  - Improved cellular health & viability
- No reliance on HDR repair
  - No donor template for base conversion
  - High editing efficacy in non-dividing cells

Horizon's Pin-point™ base editing
- Deaminase unit aptamer recruited
- Shorter enzyme units for amenable packaging
- Locus specific controls makes for multi-functional technology

FIGURE 3

Multiplex gene editing in T cell engineering.



Results are based on next generation sequencing from n=3 independent experiments with 3 different operators, 3 donors, and a total of 6 technical replications. Surface marker KO: B2M, CD52 and TRAC results shown in unstimulated condition, PD-1 in stimulate condition.

situations – especially when considering the solid tumor market, where there are many issues in relation to checkpoint inhibitors. We are very interested in how multiplex editing can benefit the immuno-oncology space.

Testing was performed with four major targets: one related to MHC 1, one related to how you would apply the therapy in the first place – i.e., TRAC, which is related to the TCR receptor to stop graft-versus-host disease – and checkpoint inhibitors.

We applied this in a multiplex setting with four different targets. As can be seen in Figure 3A, the target base responsible for the phenotype change is highly edited. The target base transition was also highly pure and predictable – in this particular example the transition is C to G (grey to maroon in Figure 3A).

In all examples, 70–90% C to T conversion was observed, which created a cascade effect leading to 70-90% protein knockout (Figure 3B). In the total population, around 50% of targets achieved quadruplex knockout, where all of the four targets are removed. This was confirmed by flow cytometry.

Interesting results were also seen when looking at the DNA (Figure 3C) to see how many abnormal events were occurring at the locus of each of the target sites. When base editing technology was compared to Cas9 wild-type first-generation CRISPR technology, a stark difference is seen regarding indel formation. This was an anticipated result, as base editing does not rely on double strand breaks as Cas9 does.

In summary, base editing was shown to be highly efficient, and readily amenable to robust multiplexing, with minimal evidence of double strand breaks.

Chimeric antigen receptor (CAR) T cell engineering

The next important question is: does this technology work in a therapeutic setting? In this study, base editing was applied in a lentiviral CAR method (Figure 4). An anti-CD9 CAR, similar to Yescarta and Keytruda, was added and applied in an *in vitro* tumor cell killing setting. CARs were generated, and CAR-positive T cells can be seen in Figure 4A. This population was highly edited, as can be seen in a pie chart representing flow cytometry readouts in Figure 4B, which shows that PC2M, TRAC, CD52 and PDCD1 are not present on the surface for over 50% of the population.

This population was then reviewed in a tumor cell killing scenario (Figure 4C). It was observed that the CAR population, whether

DOI: 10.18609/ioi.2022.005

edited or unedited, had the same high efficacy in terms of tumor cell killing.

These results demonstrate that this technology is highly amenable to multiplex editing and also maintains biological function, which is a minimum requirement to apply it in a therapeutic setting.

Multiplex gene editing effects on T cell engineering

Another area Horizon is particularly interested in is how cells are affected by different gene editing technologies. From our original studies comparing base editing versus classic CRISPR technologies, we found that Cas9 and base editing gave broadly similar results regarding T cell viability when adding one target (Figure 4A). When the number of targets is increase, it becomes evident that the double strand breaks affect cell health.

This experiment was originally performed in the quadruplex format discussed above, then expanded into an increasing quantity of targets (Figure 5B). When using base editing, the structure stays relatively flat. With Cas9 a negative correlation is seen, representing a damage response every time a target is added. When five targets is reached, as on the right of Figure 5B, this corresponds to a minimum of ten break site locations in the genome.

When comparing Cas9 and base editing, the result is a twofold increase in the quantity of cells. For a complex allogeneic therapy this is a pertinent result, as to reach the same quantity of cells, the Cas9 T cells will need to be expanded more and more in order to reach the quantity required for an adequate therapy. Furthermore, five targets may only be the tip of the iceberg when considering complex therapies in the solid tumor space.

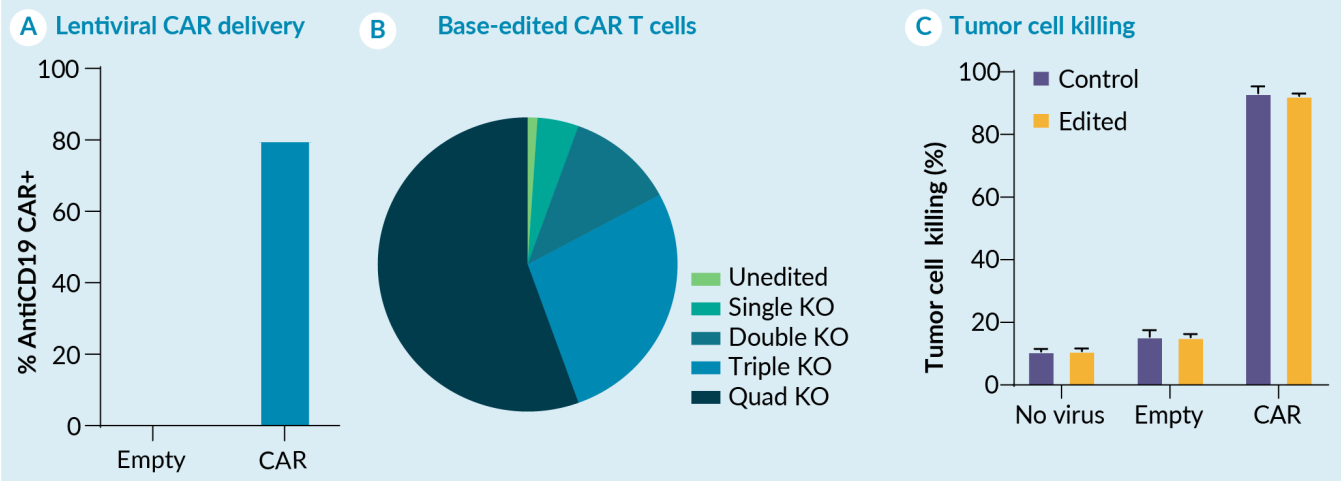
Ultimately, base editing provides higher viability proliferation and yield when multiplexing. When considering singleplexing, the main advantage is the lack of double strand breaks – but for more complex therapies, base editing provides further significant advantages in T cell engineering.

Base editing versus double-break technology: safety considerations

Gene editing is a rapidly evolving space, and safety is another key consideration. As shown in Figure 6, off-target site editing, target site translocations in multiplex editing, and

FIGURE 4

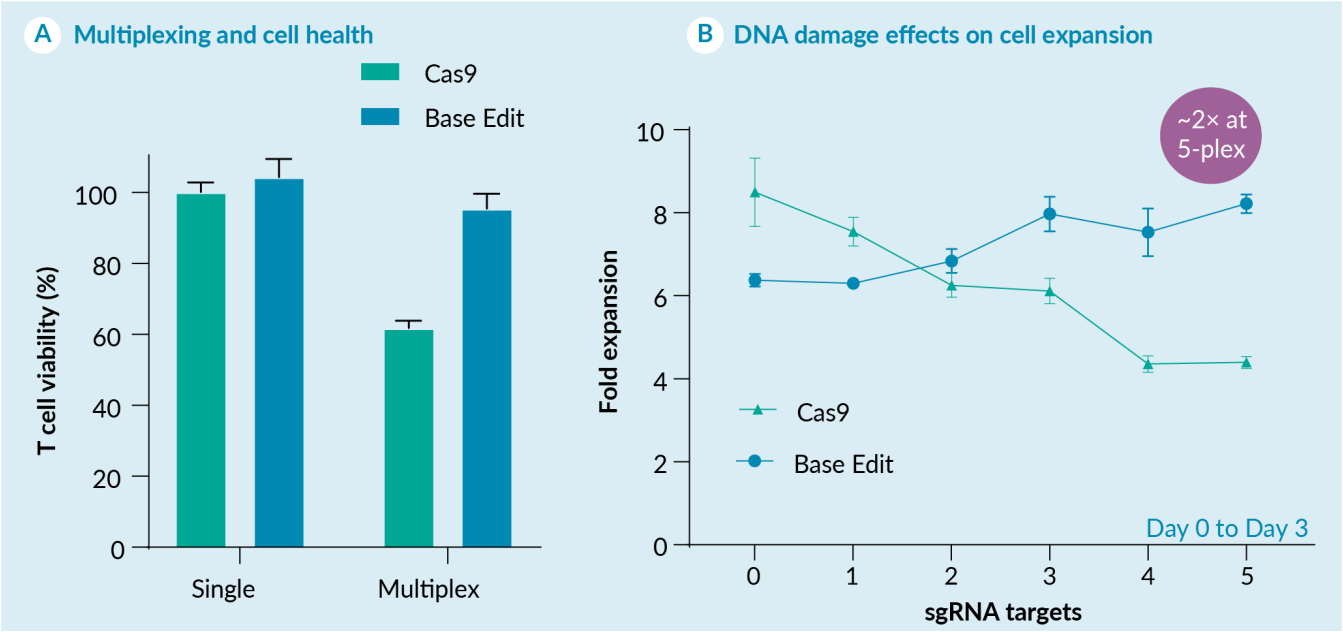
CAR T-cell engineering.



Utility of the base editing for functional *in vitro* tumour cell killing. (A) Percentage of CAR positive cells found in the transduced population, versus the lentiviral (empty) control. (B) Multiplex base editing profile of the CAR positive T cells. (C) Functionality of the edited and unedited cells in tumor cell killing; displaying non transduced, a lentiviral (empty) control, and the lentivirus containing the antiCD19 CAR.

FIGURE 5

Multiplexing and cell health.



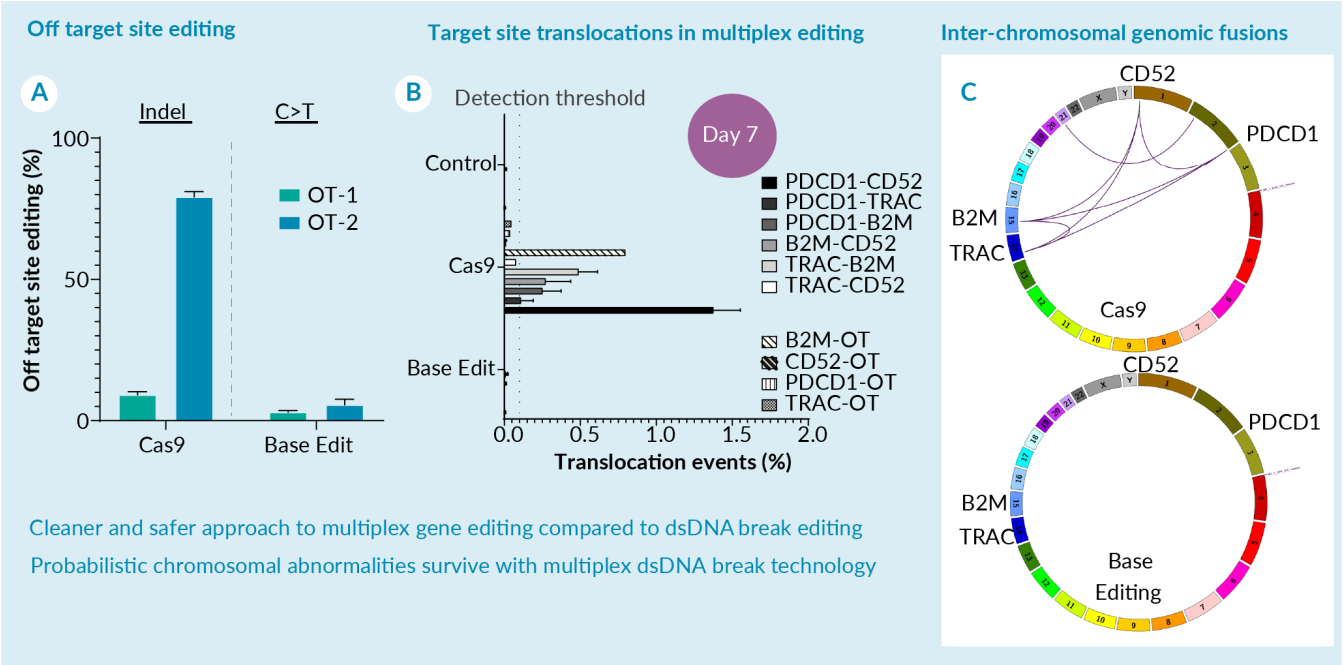
inter-chromosomal genomic fusions were studied.

Of the four guides tested to date, two off-target sites were observed using base editing, compared to ~70 for Cas9 wild type (Figure 6A). These may be non-consequential changes, such as intergenic regions in inactive genes, but could still prove problematic as the number of targets increases.

Figure 6B shows highly predictable translocation sites between different loci in the genome. For example, the black section of

FIGURE 6

Base editing versus Cas9 safety.



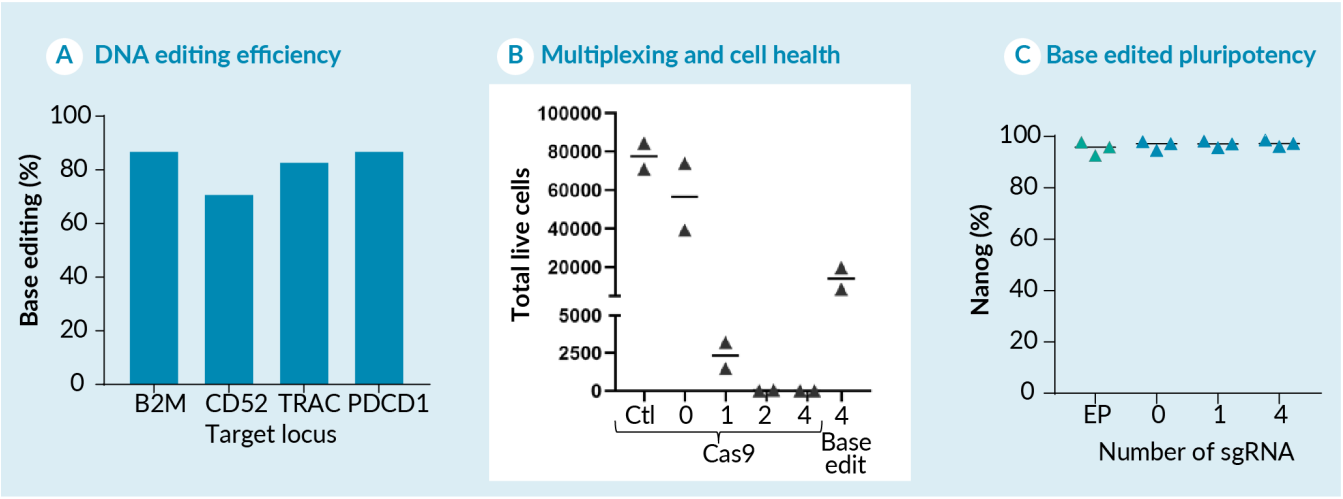
Cleaner and safer approach to multiplex gene editing compared to dsDNA break editing

Probabilistic chromosomal abnormalities survive with multiplex dsDNA break technology

DOI: 10.18609/ioi.2022.005

FIGURE 7

iPSC health following multiplex base editing.



the chart is looking at a ddPCR quantitative readout from PCD1 to CD52, which are found on completely different chromosomes. Between these two particular examples an extremely large translocation event percentage is seen – around 1 in 80 cells. When this is considered in a multiplex format (with for example four different targets) this will build up to a slightly higher percentage across the population. This could cause problems for future therapies that are using double strand break technology in a multiplex format.

Perhaps the most interesting factor is that the results shown in Figure 6B represent day 7 of the cell culture. This is not artificially inflated data from the first two hours post-delivery, or very soon after editing – this is what has survived in the culture and it is proliferating quite readily; in other words, completely surviving cells. For autologous or allogeneic therapy, a common timeframe is 5 and 10 days after the initial draw, so this is within the timeframe in which the therapy may be transferred to the patient. Whether these translocations are drastic or problematic is still to be seen, but importantly, this is not an area of concern for base editing.

We also further investigated chromosomal genomic fusions, and generated capture assays to see if they were being underestimated (Figure 6C). All indications point to the fact that this was underestimated. Again, similar results are not observed with base editing.

iPSC health after multiplex base editing

Another cell type generating interest in the immuno-oncology space is induced pluripotent stem cells (iPSCs). Potentially, iPSCs can be turned into T cells, NK cells, or even retinal neurons. As will be demonstrated, base editing works well for this purpose.

Multiplex delivery with very similar efficacies to that observed in T cells can be achieved, i.e., in the 70–90% range (Figure 7A). However, conversely to what was observed in T cells, iPSCs are not amenable to Cas9 wild type or double-strand breaks.

Figure 7B shows total live cells from the culture. On the left is the control, showing results with no manipulation, followed by an increasing quantity of guides with double-strand break technology. Even adding a single guide leads to a significant shift in the quantity of cells that survive, and the results quickly become more extreme when moving into multiplex formats. For this particular delivery method, even a duplex edit was not tolerated in iPSCs. Conversely, using four targets did not result in similar problems when base editing. A small drop was observed, but

BOX 1

Applications and benefits of base editing technology.

***Ex vivo* engineered immune cell therapies (i.e. CAR-T)**

- **Highly efficient** multiplexing gene editing
- **Complex solid tumor** gene editing approaches possible and safe
- **Adaptive** to autologous and allogeneic therapies
- **Safety:** low incidence of chromosomal translocation
- **Simpler GE workflow:** readily compatible to locus specific knockin gene delivery

***In vivo* gene therapies**

- **Monogenic diseases** (base replacement and phenotype rescue (e.g. exon skipping)
- **Polygenic diseases** (multiplexing, simultaneous editing)
- **Simpler delivery** ('one engine' with multiple effectors)

Allogeneic iPSC-derived therapies

- **Safer more streamline** universal cell line generation
- **Higher cell viability** and chromosomal health compared to ds-DNA breaks
- **Pluripotency** is retained
- **Complex gene editing strategies** (locus specific insertion and editing) possible to generate in a single step

this was not significant, and the cells were healthy and functioning.

Another important factor to consider when working with iPSCs is pluripotency (Figure 7C). The cells proved healthy, and ready to differentiate into cell types, regardless of the number of guides used. We have shown this in several different models to date.

CONCLUSION

In summary, base editing has a wide range of applications within the cell, gene, and immuno-oncology spaces, providing efficient gene editing with low incidence of chromosomal translocation (Box 1). As demonstrated above, it is a safe technology, with no translocations or chromosomal abnormalities observed to date. The workflow is simple and highly amenable to knock-in gene delivery strategies. The benefits of base editing are further enhanced by the modular properties of Horizon's Pin-point platform, which offers an advanced modular all-in-one gene editing and transfer platform, whilst preserving the core biology and integrity of the cell.

DOI: 10.18609/ioi.2022.005

ASK THE AUTHORS

John Lambourne
Research and Development Manager, Horizon Discovery

Danilo Maddalo
Group Leader, Translational Oncology, Genentech

Q Lots of work has been done on utilizing gene editing technologies to tackle hematological cancers, but what are the complications in relation to applying such technologies in solid tumors?

DM: That's a very good question. The solution is a little bit different in the case of the solid tumors, simply because of the accessibility and the homogeneity of the therapy delivery. Solid tumors are more resilient and heterogeneous to treat usually, so that makes things a bit more complicated for us.

JL: With cancer tumors, you also must consider that they are quite good at resisting penetration from T cells, sometimes referred to as being "hot" and "cold". This is another area where gene therapy applications do have a bit of an edge. It is definitely a difficulty when compared to liquid or hematological cancers, which are far more accessible.

Q Utilization of CRISPR-Cas9 technology has a very interesting role within research. What kind of roles can gene editing technologies play in drug discovery?

DM: Unlike any other technology, CRISPR-Cas9 has been adopted across the board in terms of the drug discovery process. We saw a huge impact of the technology for early target discovery. There are genetic screens which are not just limited to gene knockout, but can be extended to analysis of genetic barriers, or CRISPRa and CRISPRi approaches.

It extends to mouse models, making them much easier to generate cheaper and faster. For the first time, we can use the technology to apply functional genomics *in vivo*.

Last but not least, in the drug discovery process there is the aspect of the therapy itself. CRISPR can be used as a therapy, extending all the way down to diagnostics. It has a very broad application and impact on the drug discovery process from our point of view.

JL: Especially when you think about diagnostics; it has been a game changer in many areas.

Q As well as drug discovery, what kind of roles can gene editing play in relation to disease modeling, especially in relation to understanding cancer biology?

JL: As Danilo mentioned, we can really start applying this *in vivo*. We are utilizing certain double-strand break technologies to create *in vivo* models, which is something we could only dream of ten years ago. When you are deliberately adding a translocation which you know is deleterious, you can suddenly understand the cancer at another level. This is really interesting when it comes to seed modelling.

DM: The impact was very significant. Not only in the speed, but now instead of generating genetically engineered mouse models, we are switching to somatically engineered mouse models. We somatically engineer cells in the adult animal to generate the disease, which is actually what happens in many cases with cancer.

The quality of the impact of the editing is another aspect. For the first time, we can generate chromosomal translocations for deletions or inversions, which were very, very challenging to generate before at the somatic level. These drivers in cancer are extremely frequent, but they're relatively understudied because of the problem of generating a preclinical model. So this will help the field.

There is also the possibility of delivering a multiplex of guide RNAs. This comes with many, many limitations, but this also opens the opportunity to interrogate multiple genes at the same time in one mouse model.

Q To date, most gene editing therapies are expensive *ex vivo* treatments. What are the reasons for this, and what could make such therapies more accessible in future?

DM: Unfortunately, a limiting factor in the field is the accessibility of the therapy, although companies like Horizon are working towards making it more accessible.

It's just the beginning of the CRISPR era, so we would expect a drop in the price due to increase in the manufacturing, or novel delivery systems, which are the main bottlenecks at the moment.

Ex vivo engineering has to go through many steps of validation to make sure that no off-targets are affecting the fitness of the cells. This requires a lot of time, and is something that will probably stay within the field, but will go down in terms of price.

JL: It's definitely an interesting problem. When we think about these sorts of treatments, we skirt around the fact that these are million-dollar treatments. It is a very logistically

DOI: 10.18609/ioi.2022.005

tricky process to take cells out of someone, manipulate and put them back in. To make certain therapies more accessible, we need to change the way we utilize or apply this technology.

A lot of people are interested in iPSCs because they are ideally manufactured outside of someone, and this could potentially reduce such costs going forward.

DM: John really covered the limitations. One has to also consider that these are hopefully one-time therapies. They do not require pre-treatment or continuous treatment. The price effectiveness becomes comparable if you measure them against therapies that would require a lifetime treatment. It really depends on the type of diseases we are talking about.

Q Safety profiling is obviously a hot topic. Why is it so important, and what is needed to demonstrate that such cell and gene therapies are safe enough? What will it take to determine the technology is safe enough for the lowest severity diseases or disorders?

DM: This is probably the most relevant question now in the field. As with any new modality or therapy, the major concerns are usually safety. We cannot go with the multiplex of the guide RNAs and the Cas9 to fix many of these diseases, because that would generate catastrophic events from the karyotype point of view. We would have a lot of uncontrollable translocations or deletions that we could not even fully detect.

It will require a set of tools like base editors for example, that are proven to be a little bit safer and more precise. It requires a set of experiments *in vitro* to show that genetically we are not significantly changing the carrier type of the cells that we are treating.

There is a whole part of safety that goes beyond this technology, which focuses on the vectors that are used to deliver the therapy. Viral vectors, or any other type of delivery method, require a separate safety package. These are normal steps that require some time, but they will pay off in the long-term.

Q Is there any data available on base editing of hematopoietic stem cells (HSCs)?

JL: We have not played too much in this lab, but there is plenty out there. There are some INDs out there in relation to base editing in HSCs, so it has been quite well used.

It has not really been utilized for the more extravagant treatments so far. I think the most obvious example is sickle cell anemia, where you have a point mutation in your *HBB* gene and you are looking to upregulate fetal hemoglobin to help rescue the disease.

BIOGRAPHIES

John Lambourne
Research and Development Manager, Horizon Discovery

Dr John Lambourne is a R&D Manager in Base Editing, Cell and Gene Therapy team of PerkinElmer's Horizon Discovery business. Since joining the company in November 2018, his work has contributed towards the development and commercialisation of novel technologies for cell and gene therapy applications. He now works on driving the Pin-point™ base editing technology to its full potential in next generation therapeutics.

Danilo Maddalo
Group Leader, Translational Oncology, Genentech

Danilo Maddalo got his PhD from the Karlsruhe Institute of Technology (Germany) and trained at Memorial Sloan Kettering Cancer Center (NYC, USA) before opening his first lab at the Novartis Institute for Biomedical Research (Basel, Switzerland). He is currently a group leader at Genentech (South San Francisco, USA) where he focuses on *in vivo* pharmacology, translational research and genome editing.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

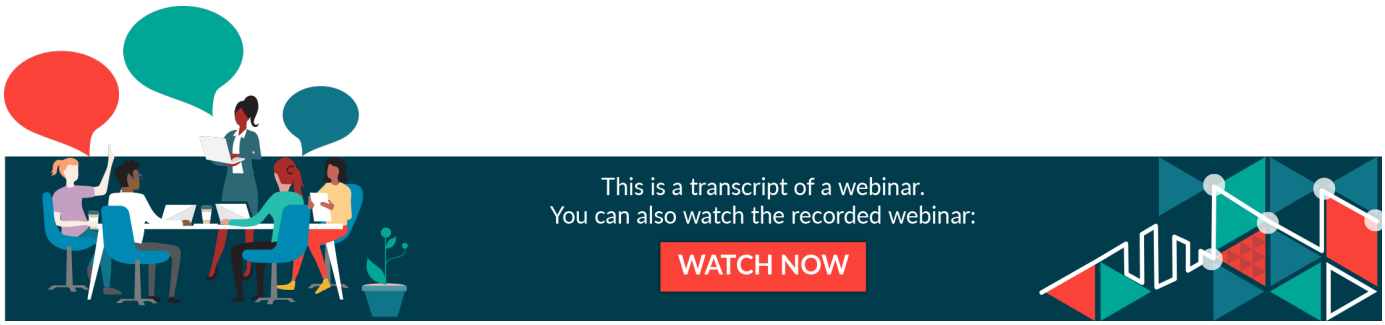
Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *This article is a transcript of a previously published webinar, which can be found here.*

Webinar published: *Nov 11 2021;* ***Publication date:*** *Jan 26 2022.*



DOI: 10.18609/ioi.2022.005

IMMUNO-ONCOLOGY INSIGHTS

ANTICIPATING IMMUNO-ONCOLOGY MODALITY/ PLATFORM DEVELOPMENT TRENDS FOR 2022

SPOTLIGHT

COMMENTARY/OPINION

BCMA targeting CAR T cells using a novel D-domain binder for multiple myeloma: clinical development update

Anand Rotte, Christopher Heery, Bradford Gliner, David Tice & David M Hilbert

Relapsed and refractory multiple myeloma patients have poor prognoses and limited treatment options even with the recent FDA approval of idecabtagene vicleucel. Idecabtagene vicleucel joins four other US FDA approved chimeric antigen receptor T cell therapies each with promising efficacy and response rates across various hematological malignancies. Notwithstanding the early success of chimeric antigen receptor T therapies, there remain many challenges that warrant further development of chimeric antigen receptor T structure and function as they relate to durability of response, T cell exhaustion due to tonic signaling, immunogenicity, manufacturing-related limitations, and incidence of serious adverse events including cytokine release syndrome and immune effector cell associated neurotoxicity syndrome. D-domain based chimeric antigen receptor T cells are a new class of structurally and functionally distinct cell therapies that represent an alternative to conventional single-chain variable fragment based chimeric antigen receptor T cells. Preclinical studies of a D-domain based targeting B cell maturation antigen (CART-ddBCMA) have demonstrated effective anti-tumor response in both *in vitro* and tumor models. These studies currently support a first-in-human clinical study of CART-ddBCMA in multiple myeloma patients. Interim Phase 1 data presented at American Society of Hematology 2021 Annual Meeting further demonstrates the promising potential of CART-ddBCMA cells in the treatment of multiple myeloma. Herein, we present an overview of chimeric antigen receptor T cell therapies including challenges in chimeric antigen receptor T therapies, advances in CAR design, structural and functional properties of novel D domain-based chimeric antigen receptor T cells, and an update on the development of CART-ddBCMA in multiple myeloma patients.

Immuno-Oncology Insights 2022; 3(1), 13–24

DOI: 10.18609/ioi.2022.003

Multiple myeloma (MM) is the third most common hematological malignancy in the USA and the world [1,2] and ranks second in the death rate for hematological malignancy in the USA [1]. This heterogenous disease is characterized by the proliferation of clonal plasma cells that secrete a monoclonal antibody called an M protein [3]. MM patients have a median age at diagnosis of 69 years and one-third of patients diagnosed are ≥75 years of age [4]. Approximately half of patients (all ages) and only a quarter of patients above age 80 survive 5 years after being diagnosed with MM [5,6].

MM is mainly treated with an induction regimen comprising a proteasome inhibitor (PIs; bortezomib), immunomodulatory drugs (IMiDs; thalidomide, lenalidomide) and dexamethasone often followed by autologous stem cell transplantation [3]. More recently, newer molecular entities including monoclonal antibodies (daratumumab, elotuzumab), second generation PIs (carfilzomib, ixazomib) and IMiDs (pomalidomide) have demonstrated clinical efficacy in the treatment of MM and have received FDA approval as combination therapy. However, despite the availability of multiple new treatments, resistance develops in some patients resulting in the relapse of the disease requiring administration of next line of treatment. Interestingly, patients who relapse quickly and need next line of treatment were reported to have shorter survival [7]. Prognosis for relapsed and refractory MM is very poor with median overall survival shown to be two-fold lower in penta-refractory patients compared to patients who failed fewer than 3 lines of treatment (5.6 months vs 11.2 months respectively) [8]. Thus, there is an unmet need to develop durable treatment options for the treatment of relapsing and refractory MM [9]. Chimeric antigen receptor T (CAR T) cell therapies have demonstrated promising clinical efficacy in hematological cancers and to date, five therapies have been approved for different types of hematological cancers including multiple myeloma (Table 1). The current article summarizes the advances in CAR-T cells, challenges, the potential of d-domain based CARs and their application in the treatment of multiple myeloma.

CAR-T CELLS

CAR T cells are engineered to eradicate tumor cells and have proven effective in the treatment of several hematological malignancies [10–12]. The first step in generating CAR T cells is to collect a patient's or an allogeneic donor's T cells using a process known as apheresis. T-cells are then isolated from the apheresis product, genetically modified to express a CAR via electroporation, viral transduction, or CRISPR-CAS methods. CAR T cells are then further expanded *ex vivo* before returning the CAR T product to the patient.

Each CAR comprises an extracellular antigen-binding domain fused to a hinge region which in turn is fused to a transmembrane domain, and one or more intracellular signaling domain. Each structural component has a distinct function, and the evolution of these components has defined the progress of CARs.

PROGRESS IN CAR-T CELL DESIGN

The first-generation CAR, which consisted of an extracellular antigen-binding single-chain variable fragment (scFv) fused to a transmembrane region and the intracellular signaling domain, CD3ζ was pioneered by Zelig Eshhar and his colleagues [13–16]. The second generation of CARs were developed to address the issues of failed expansion and persistence commonly associated with first generation of CAR T cells, include a CD28 or 4–1BB co-stimulatory domain located between the transmembrane and CD3 domains. To improve the persistence and proliferation, CARs were developed with both co-stimulatory domains (CD28 and 4–1BB) located between the transmembrane and CD3 domains. Further advancement in CAR T cells was the development of T cells redirected for universal cytokine-mediated killing

DOI: 10.18609/ioi.2022.003

TABLE 1

Commercially available CAR-T cell therapies (current as of December 2021).

CAR-T therapy	Target	Indication
Axacabtagene ciloleucel	CD19	Relapsed and refractory B-cell lymphoma including DLBCL and follicular lymphoma after 2 or more lines of therapy
Brexucabtagene autoleucel	CD19	Relapsed and refractory mantle cell lymphoma
Tisagenlecleucel	CD19	Children and young adults (up to 25 years of age) with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse Adults with relapsed or refractory B-cell lymphoma after 2 or more lines of systemic therapy
Lisocabtagene maraleucel	CD19	Relapsed and refractory B-cell lymphoma including DLBCL after 2 or more lines of therapy
Idecabtagene vicleucel	BCMA	Multiple myeloma after 4 or more lines of therapy

(TRUCKs), which include a transgene that is designed to stimulate the secretion of a cytokine such as interleukin-12 upon CAR activation. TRUCKs were developed to improve CAR T cell response in tumors with phenotypic diversity [17,18].

CLINICAL STUDIES

Early clinical trials demonstrated that administration of CAR-expressing T cells can lead to complete remission in certain hematological malignancies including relapsed or refractory B-cell lymphoma [19,20]. Tisagenlecleucel or tisa-cel, the first CAR T cell therapy to be approved by FDA established an overall response rate (ORR) of 83% and complete response (CR) rate of 63% in pediatric and young adults with relapsed or refractory (r/r) B-cell acute lymphoblastic leukemia (ALL) and an ORR of 50% and CR of 32% in adults with relapsed or refractory (r/r) Diffuse large B-cell lymphoma (DLBCL) [21]. Axi-cel (axicabtagene ciloleucel) treatment was shown to have an ORR of 72% and CR rate of 51% in adults with relapsed or refractory large B-cell lymphoma and an ORR of 89% and CR rate of 62% in adults with relapsed and refractory follicular lymphoma [22]. In a similar patient cohort (adults with relapsed and refractory large B-cell non-Hodgkin's lymphoma including DLBCL), liso-cel (lisocabtagene maraleucel) had an ORR of 73% and CR rate of 53% [23]. Efficacy of brexu-cel (brexucabtagene autoleucel) was studied in adults with relapsed and refractory mantle cell lymphoma and the ORR was 80% and CR rate was 55% [24].

Two CAR T therapies, ide-cel (idecabtagene vicleucel) and cilta-cel (ciltacabtagene autoleucel) were studied in multiple myeloma patients. Ide-cel is approved by US FDA for the treatment of adult patients with relapsed or refractory multiple myeloma after four or more prior lines of therapy, including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody [25] whereas cilta-cel is awaiting the decision on the Biologics License Application (BLA). In the pivotal trials, ide-cel recorded an ORR of 72% and CRR of 28% [25] and cilta-cel had an ORR of 95% and CR rate of 60% [26].

However, currently available CAR T cell therapies are limited by concerns of durability of efficacy, safety, and production issues (Box 1). Literature review of relapse rates seen with CD19 targeting CAR T cell therapies showed that nearly 30–80% of responding patients had a relapse of the disease within 1 to 7 months [27], and nearly 20% of multiple myeloma patients treated with BCMA targeting CAR T cell therapies were shown to develop resistance [28–30]. Cytokine release syndrome (CRS) and neurotoxicity, adverse events known to be associated with therapies activating effector immune cells are commonly reported with CAR T cell therapies.

BOX 1

Challenges to CAR-T cell therapy.

1. **Tonic signaling leading to early exhaustion of CAR-T cells**
2. **Anti-drug responses against CAR-T cells**
3. **Durability of efficacy**
4. **Low expression of CAR+**
5. **High inter-patient variation in CAR⁺ cells in final product**
6. **Incidence of adverse events including CRS and ICANS**

In the pivotal studies, the incidence of all grade CRS events ranged from 42 to 95% and grade ≥3 events ranged from 2 to 22%. The incidence of all grade neurotoxicity events ranged from 18 to 64% and grade ≥3 events ranged from 3 to 28% [26,31–34]. In the real-world study, the incidence of CRS and neurotoxicity events in patients treated with axi-cel or tisa-cel was reported to be 85% (all grade) and 8% (grade ≥3) for CRS and 28% (all grade) and 10% (grade ≥3) for neurotoxicity [35].

The use of scFV in the CAR T cell has been shown to be associated with antigen-independent clustering of CAR-scFVs resulting in early exhaustion and decreased anti-tumor activity of CAR T cells [36–38]. Further, murine-derived scFVs can be immunogenic when used in humans and may result in treatment failure and/or limit the success of redosing the patients [39,40]. Finally, expression of CAR transgene in the final product can be less than 50% and is also known to have considerable inter-patient variability (15–70%), which can affect the efficacy and safety of the therapy [41–49]. Multiple approaches have been proposed to address the challenges to CAR T cell therapy including dose fractionation and use of alternatives to scFVs such as ankyrin repeats [50], adnectins [51], thermo-stable DNA-binding proteins [52], affibodies [53] and D-domain proteins [54]. Among the alternatives to scFVs, D-domain proteins are shown to have unique advantages and have demonstrated efficacy in *in vivo* tumor models [55].

BCMA AS A TARGET FOR MM

While CD19 has proven to be an effective target for a number of B cell lymphomas, the malignant plasma cells in MM are known to express little or no CD19 on their surface thereby rendering CD19-specific CAR T cells ineffective in the relapsed and refractory multiple myeloma (RRMM) setting [56,57]. In contrast, the transmembrane receptor, B-cell maturation antigen (BCMA, CD269), is uniquely associated with normal plasma cells and diseased cells within the plasma cell lineage including monoclonal gammopathy of undetermined significance (MGUS), smoldering myeloma, and MM [56,57]. The importance of BCMA as a therapeutic target is further supported by the presence of elevated soluble BCMA in serum of patients with plasma cell diseases, the levels of which are a surrogate biomarker that correlates with tumor burden [57–61].

The association of BCMA with plasma cell diseases has led to the development of several classes of BCMA-targeting therapies. Among the earliest therapies to advance were antibody drug conjugates (ADC) and bi-specific engager proteins (bsAbs) [62]. To date, the ADC belantamab mafodotin is the only drug in this class to receive FDA approval in RRMM with an overall response rate (ORR) of 34%, a median PFS of 3.9 months, and a one-year overall survival (OS) probability of 53% in the high dose (3.4 mg/kg every three weeks) [62–64]. Although belantamab mafodotin is clearly an important therapeutic option for the treatment of RRMM, its safety and efficacy profile does not compare

DOI: 10.18609/ioi.2022.003

favorably with many of the BCMA CAR Ts currently in development including the only FDA approved BCMA targeting CAR T (Idecel) with a 72% ORR and median duration of response of 11.3 months [32].

It remains critical that emerging BCMA CAR T therapies address the lingering safety and efficacy challenges seen among earlier BCMA targeting therapies [25,65]. Towards that end, the recent BLA submission for the BCMA-targeting CAR T, cilta-cel included encouraging data supporting an ORR of 95% and CR rate of 60% in RRMM [26]. Future CAR T cells therapies must continue to enhance their respective structural and functional properties to further improve BCMA CAR T safety and efficacy profiles. Successful development of new CAR Ts may open such therapies to patients who are not currently eligible for cell therapy due to a poor clinical status that can limit their ability to tolerate potential toxicities associated with CAR T therapies. Innovative strategies are also needed to address manufacturing limitations and minimize variability in CAR expression across patient populations. The novel BCMA-targeting D-domain based CAR T therapy discussed in the following sections was designed with an objective of addressing the challenges of CAR T cell therapy and improve efficacy while maintaining an acceptable safety profile.

D-DOMAIN PROTEINS

D-domains are synthetic proteins (~8 kDa) with approximately one-third the mass of the scFvs typically found in conventional CARs. D domains can be further distinguished structurally as each forms a triple α-helical bundle stabilized by a hydrophobic core with no disulfide bonds or N-linked glycosylation sites [54]. All target binding D-domains are derived from a parental D domain referred to as α3D [66,67] in which specific residues have been randomized. The ability to generate, deimmunize and functionally optimize the biological properties of novel antigen-binding D-domains affords the opportunity to enhance and/or replace the binding specificities found in a broad spectrum of native soluble proteins, antibodies and cellular receptors.

IDENTIFICATION OF DDBCMA & CONSTRUCTION OF CAR T-DDBCMA

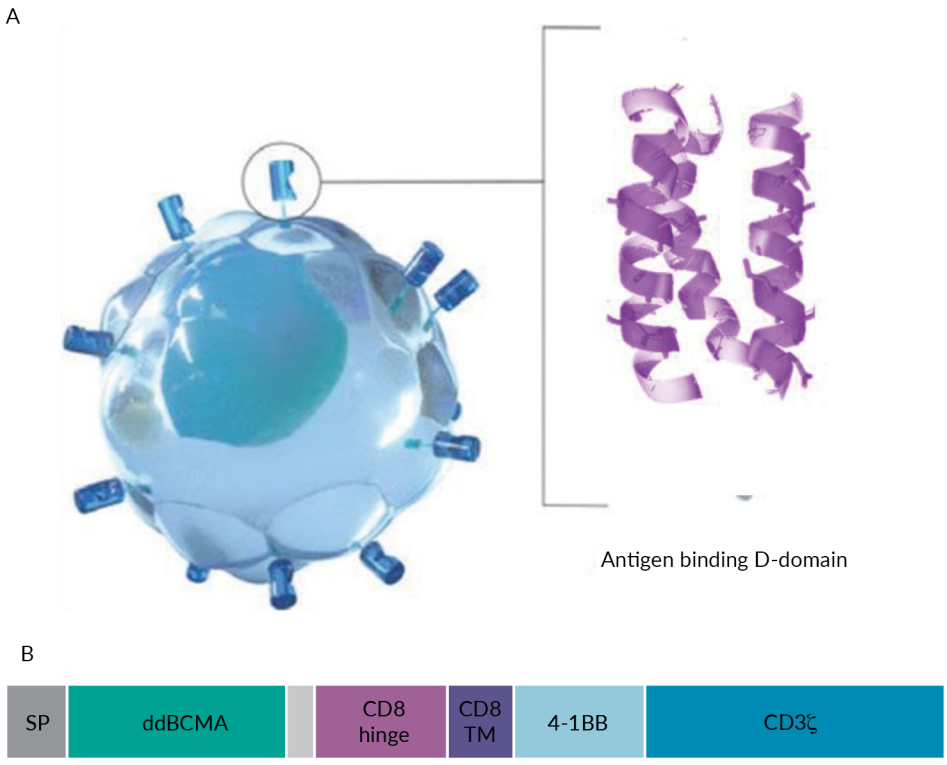
A BCMA binding D-domain (ddBCMA) was identified in a library of randomized α3D sequences using standard phage-display technologies. Subsequent site-directed mutagenesis to enhance target affinity and minimize immunogenicity resulted in a 73 amino acid D domain with nanomolar affinity for human BCMA. CAR was constructed by fusing ddBCMA to a linker followed by a CD8a transmembrane region, the 4–1-BB and CD3-zeta intracellular signaling domains (Figure 1). The resulting CAR construct was cloned into a lentiviral vector for gene transfer to T cells.

PRECLINICAL EXPERIENCE

The ability of CAR T-ddBCMA to activate antigen-specific intracellular signaling and cytolytic function was studied using *in vitro* experiments and *in vivo* tumor models [68]. Across a series of *in vitro* assays, CAR T-ddBCMA displayed reproducible BCMA-dependent NFAT signaling, cytokine (IL-2, IFN-λ) secretion, and specific killing of BCMA-expressing tumor cell lines. *In vivo*, mouse-human xenograft models further demonstrated BCMA-dependent activity as evidenced by the ability of CAR T-ddBCMA to eradicate BCMA-expressing tumors within 2 weeks of single administration of CAR T-ddBCMA. Parallel safety assessments indicated that CAR T-ddBCMA did not significantly impact mouse body weights nor did CAR T-ddBCMA produce any attributable histopathological findings [68]. Taken together, the preclinical safety and efficacy profiles for CAR T-ddBCMA supported early-stage clinical development of this therapy in RRMM.

FIGURE 1

(A) CART-ddBCMA cells showing the antigen binding D-domain. (B) Vector construct for ddBCMA CAR



CLINICAL EXPERIENCE

CAR T-ddBCMA cells are currently investigated in a Phase 1, multi-center, open label, dose escalation trial. The study is enrolling subjects with relapsed and refractory MM who have received at least three prior regimens, including a proteasome inhibitor, an immuno-modulatory agent, and a CD38 antibody or are triple refractory. In line with previous anti-BCMA CAR T studies [26,32], pre-specified BCMA expression level on tumor cells is not a requirement for enrollment in the study. The primary endpoint of the study is incidence of adverse events (AEs), including dose-limiting toxicities (DLTs). In addition, quality and duration of clinical response assessed according to the International Myeloma Working Group (IMWG) uniform response criteria, minimal residual disease (MRD), and progression-free and overall survival are evaluated.

Peripheral blood mononuclear cells are collected from subjects meeting the study inclusion criteria via leukapheresis and sent to a central facility for the production and cryopreservation of CAR T-ddBCMA cells. Before treatment with CAR T cells, subjects undergo lymphodepletion (fludarabine [30 mg/m^2] and cyclophosphamide [300 mg/m^2] treatment daily for three days) followed by CAR T-ddBCMA cells administration as a single infusion at two dose levels, 100 million (DL1) and 300 million (DL2) CAR$^+$ T cells.

Interim results of the study were presented at American Society of Hematology Annual Conference, 2021 [69]. At the time of last data-cut, 26 subjects (median age 66.0 [range: 44–76]) were enrolled and 24 received CAR T-ddBCMA cell therapy (Table 2). Twenty-two subjects were evaluable for initial safety and 19 subjects were evaluable for clinical response analysis. Median follow-up time for the subjects was 283 days (~9 months; range:

DOI: 10.18609/ioi.2022.003

115–640 d). Median prior lines of therapy in the subjects was five (range: 3–16); 20 subjects were "triple-refractory" and 17 subjects were "penta-refractory"; 17 subjects had high-risk cytogenetics. Tumor burden was ≥50% bone marrow plasma cells in ten subjects and nine subjects had extramedullary disease at baseline.

Eighteen subjects received treatment at DL1, and six subjects received at DL2. Median CAR$^+$ expression in total CD3$^+$ T cells was 74% and inter-patient variation in CAR+ expression was relatively low (range: 61–87%). CAR$^+$ T cell expansion was observed in all evaluable subjects by ddPCR and peak expansion was noted within 2 weeks. Safety was evaluable in subjects with at least 1 month of follow-up (N = 22). The treatment was well tolerated by the subjects and majority (N = 21/22) of cytokine release syndrome (CRS) events were grade 2 or less. One subject in the DL2 cohort had grade 3 CRS. Median time to onset of CRS was 1 day at both dose levels, and median duration of the first CRS was 6.5 days in DL1 and 4.5 days in DL2. Four subjects, three in DL1 (two, grade≤2; one , grade≥3) and one subject (grade≥3) in DL2 cohort had immune effector cell associated neurotoxicity syndrome (ICANS). Median time to onset of ICANS was 3 days in DL1 group and 6 days in DL2 group, and median duration of ICANS was 8 days in DL1 and 17 days in DL2. CRS was managed with tocilizumab with or without dexamethasone and ICANS were managed with anakinra plus dexamethasone. At the time of presentation, all CRS and ICANS events were resolved without sequalae.

Recently, BCMA expression was reported in neurons and astrocytes in the basal ganglia of a patient [70] and CAR T cell treatment has been shown to be associated with delayed onset (~3 months) of progressive movement disorders with features of Parkinson's disease, an 'on-target off-tumor' effect. At the time of last data-cut, no Parkinsonian symptoms or delayed onset neurological events have been observed in any patient after treatment with CAR T-ddBCMA. However, subjects will be monitored for delayed onset neurological changes in the study and increased vigilance will be implemented.

Efficacy was evaluable in subjects with at least 3 months of follow up (N = 19). Clinical response to CAR T-ddBCMA treatment was very encouraging and all 19 evaluable

TABLE 2

Patient demographics*.

Characteristics	Dose level 1 100 million CAR-T (n=18)	Dose level 2 300 million CAR-T (n=6)	Total (n=24)
Age, median (min-max)	69 (44-76)	60 (52-65)	66 (44-76)
Gender	8 Male (44%) 10 Female 56%)	5 Male (83%) 1 Female (17%)	13 Male (54%) 11 Female (46%)
BMPC >50%	6/18 (33%)	4/6 (67%)	10/24 (42%)
Extra-medullary disease	6/18 (33%)	3/6 (50%)	9/24 (38%)
High-risk cytogenetics per IMWG	13/14 (93%)**	4/5 (80%)**	17/19 (89%)**
Prior Lines of Therapy, median (min-max)	5 (3-9)	4 (3-16)	5 (3-16)
Triple refractory	15/18 (83%)	5/6 (83%)	20/24 (83%)
Penta refractory	13/18 (72%)	4/6 (67%)	17/24 (71%)
IgG myeloma IgA myeloma Light chain only	10 3 4	5 0 1	15 3 5

BMPC: Bone marrow plasma cell; HSCT: Hematopoietic stem cell transplant; IMWG: International Myeloma Working Group.
*Data presented at ASH2021 Annual Meeting [69]
†Penta-refractory patients are refractory to bortezomib, carfilzomib, daratumumab, lenalidomide, and pomalidomide.
**Some subjects were not evaluable or data were not available at time of data cut.

TABLE 3

Latest clinical findings from CART-ddBCMA Phase 1 study*.

	N=19 (efficacy evaluable subjects)**
Objective response rate, n (%)	19 (100%)
Stringent complete response, n (%)	10 (63%)
Complete response, n (%)	3 (14%)
Very good partial response	3 (14%)
Partial response, n (%)	3 (14%)
$MRD^{(-)}$ (15 evaluable subjects)	7 at 10^{-6}, 5 at 10^{-5}, and 2 at 10^{-4}
CAR^+ cells in product, median % (range)	74% (61%-87%)
CRS, n (%)	22 (100%)
Grade 1-2, n (%)	21 (95%)
Grade≥3, n (%)	1 (5%); seen in DL2 cohort
ICANS, n (%)	4 (18%)
Grade 1-2, n (%)	2 (9%)
Grade≥3, n (%)	2 (9%)

*Data presented at ASH2021 Annual Meeting [69]
**Efficacy was evaluable in subjects with at least 3 months of follow-up.

subjects (100%) demonstrated clinical response per IMWG criteria (Table 3). Ten subjects had stringent complete response (sCR), three had CR, three had very good partial response (VGPR) and three had PR. Of the subjects (N = 15) with mean residual disease (MRD) data, seven were $MRD^{(-)}$ at 10^{-6}, five at 10^{-5} and two at 10^{-4}. Interestingly, $MRD^{(-)}$ status was seen at month 6 or later in most cases indicating the deepening of response with time. More importantly, the responses to CAR T-ddBCMA treatment deepened over time. Of the eight subjects with follow-up over 12 months, five continued to have sCR after 12 months including three subjects with follow-up over 20 months, supporting the durability of CAR T-ddBCMA efficacy.

TRANSLATIONAL INSIGHTS

CAR T cells have enormous potential in the treatment of cancer, especially hematological cancers including multiple myeloma. However, there is scope for further improvement in the design of CAR T cells to address tonic signaling, antigenicity, low CAR^+ transfection rate and inter-patient variations in CAR^+ transfection rate. BCMA targeting D-domain based CAR T cells (Figure 1) have shown promising results in the preclinical and clinical studies with >70% CAR+ transfection rate, negligible inter-patient variation in CAR^+ expression and high response rates possibly due to lower tonic signaling and antigenicity (Table 4). Further studies are currently planned to confirm the clinical benefits CAR T-ddBCMA cells.

TABLE 4

Features of CART-ddBCMA.

Feature	Impact
BCMA as the target	▶ Ideal target for multiple myeloma
D-domains as target antigen-binding domain	▶ Designed to have low immunogenicity ▶ Low tonic signaling ▶ Low inter-patient CAR^+ cell variation ▶ Improved production efficiency
Inclusion of 4-1-BB intracellular signaling domains	▶ Durable efficacy
Lentiviral construct	▶ Reliable gene transfer and transfection rate

DOI: 10.18609/ioi.2022.003

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J. Clin*. 2021; 71, 7–33.
2. Sun H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin*. 2021; 71, 209–249.
3. Palumbo A, Anderson K. Multiple myeloma. *N Engl J. Med*. 2011; 364, 1046–1060.
4. Hari P, Romanus D, Luptakova K *et al*. The impact of age and comorbidities on practice patterns and outcomes in patients with relapsed/refractory multiple myeloma in the era of novel therapies. *J. Geriatr Oncol*. 2018; 9, 138–44.
5. Cancer Stat Facts: Myeloma. *NIH: NCI*. https://seer.cancer.gov/statfacts/html/mulmy.html (Accessed Jan 2022).
6. Myeloma statistics. CancerResearchUK. (Accessed Jan 2022).
7. Majumder MM, Silvennoinen R, Anttila P *et al*. Identification of precision treatment strategies for relapsed/refractory multiple myeloma by functional drug sensitivity testing. *Oncotarget*. 2017; 8, 56338–56350.
8. Gandhi UH, Cornell RF, Lakshman A *et al*. Outcomes of patients with multiple myeloma refractory to CD38-targeted monoclonal antibody therapy. *Leukemia* 2019; 33, 2266–2275.
9. Nijhof IS, van de Donk N, Zweegman S, Lokhorst HM. Current and New Therapeutic Strategies for Relapsed and Refractory Multiple Myeloma: An Update. *Drugs*. 2018; 78, 19–37.
10. Huang R, Li X, He Y *et al*. Recent advances in CAR-T cell engineering. *J. Hematol. Oncol*. 2020; 13, 86.
11. Maus MV, June CH. Zoom Zoom: racing CARs for multiple myeloma. *Clin. Cancer Res*. 2013; 19, 1917–1919.
12. Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat. Rev. Cancer*. 2021; 21, 145–161.
13. Rosenberg SA, Packard BS, Aebersold PM *et al*. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. *N. Engl. J. Med*. 1988; 319, 1676–1680.
14. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc Natl Acad Sci USA*. 1989; 86, 10024–10028.
15. Gross G, Gorochov G, Waks T, Eshhar Z. Generation of effector T cells expressing chimeric T cell receptor with antibody type-specificity. *Transplant. Proc*. 1989; 21(1 Pt 1), 127–130.
16. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc. Natl Acad. Sci. USA*. 1993; 90, 720–724.
17. Chmielewski M, Hombach AA, Abken H. Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. *Immunol. Rev*. 2014; 257, 83–90.
18. Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. *Expert Opin. Biol. Ther*. 2015; 15, 1145–54.
19. Firor AE, Jares A, Ma Y. From humble beginnings to success in the clinic: Chimeric antigen receptor-modified T-cells and implications for immunotherapy. *Exp. Biol. Med. (Maywood)*. 2015; 240, 1087–1098.
20. Styczyński J. A brief history of CAR-T cells: from laboratory to the bedside. *Acta. Haematol. Pol*. 2020; 51, 2–5.
21. Package insert – Kymriah®. *FDA*. 2020.
22. Package insert – Yescarta®. *FDA*. 2021.
23. Package insert – Breyanzi®. *FDA*. 2021.
24. Package insert – Tecartus®. *FDA*. 2021.
25. Package insert – Abecma®. *FDA*. 2021.
26. Berdeja JG, Madduri D, Usmani SZ *et al*. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. *Lancet*. 2021; 398, 314–324.
27. Xu X, Sun Q, Liang X, Chen Z *et al*. Mechanisms of Relapse After CD19 CAR T-Cell Therapy for Acute Lymphoblastic Leukemia and Its Prevention and Treatment Strategies. *Front Immunol*. 2019; 10, 2664.
28. Teoh PJ, Chng WJ. CAR T-cell therapy in multiple myeloma: more room for improvement. *Blood Cancer J*. 2021; 11, 84.
29. Da Via MC, Dietrich O, Truger M *et al*. Homozygous BCMA gene deletion in response to anti-BCMA CAR T cells in a patient with multiple myeloma. *Nat. Med*. 2021; 27, 616–619.

30. Brudno JN, Maric I, Hartman SD *et al.* T Cells Genetically Modified to Express an Anti-B-Cell Maturation Antigen Chimeric Antigen Receptor Cause Remissions of Poor-Prognosis Relapsed Multiple Myeloma. *J. Clin. Oncol*. 2018; 36, 2267–2280.

31. Abramson JS, Palomba ML, Gordon LI *et al.* Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet*. 2020; 396, 839–852.

32. Munshi NC, Anderson LD, Jr., Shah N *et al.* Idecabtagene Vicleucel in Relapsed and Refractory Multiple Myeloma. *N. Engl. J. Med*. 2021;384,705–16.

33. Neelapu SS, Locke FL, Bartlett NL *et al.* Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N Engl. J. Med*. 2017; 377, 2531–44.

34. Schuster SJ, Bishop MR, Tam CS *et al.* Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *N. Engl. J. Med*. 2019; 380, 45–56.

35. Sesques P, Ferrant E, Safar V *et al.* Commercial anti-CD19 CAR T cell therapy for patients with relapsed/refractory aggressive B cell lymphoma in a European center. *Am. J. Hematol*. 2020; 95, 1324–1333.

36. Long AH, Haso WM, Shern JF *et al.* 4–1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. *Nat. Med*. 2015; 21, 581–590.

37. Ajina A, Maher J. Strategies to Address Chimeric Antigen Receptor Tonic Signaling. *Mol Cancer Ther*. 2018; 17, 1795–1815.

38. Calderon H, Mamonkin M, Guedan S. Analysis of CAR-Mediated Tonic Signaling. *Methods Mol Biol*. 2020; 2086, 223–236.

39. Lamers CH, Willemsen R, van Elzakker P *et al.* Immune responses to transgene and retroviral vector in patients treated with ex vivo-engineered T cells. *Blood* 2011; 117, 72–82.

40. Wagner DL, Fritsche E, Pulsipher MA *et al.* Immunogenicity of CAR T cells in cancer therapy. *Nat. Rev. Clin. Oncol*. 2021; 18, 379–393.

41. Jiang H, Liu L, Guo T *et al.* Improving the safety of CAR-T cell therapy by controlling CRS-related coagulopathy. *Ann. Hematol*. 2019; 98, 1721–1732.

42. Hu Y, Wu Z, Luo Y *et al.* Potent Anti-leukemia Activities of Chimeric Antigen Receptor-Modified T Cells against CD19 in Chinese Patients with Relapsed/Refractory Acute Lymphocytic Leukemia. *Clin. Cancer Res*. 2017; 23, 3297–3306.

43. Wang CM, Wu ZQ, Wang Y *et al.* Autologous T Cells Expressing CD30 Chimeric Antigen Receptors for Relapsed or Refractory Hodgkin Lymphoma: An Open-Label Phase I Trial. *Clin. Cancer Res*. 2017; 23, 1156–1166.

44. Magnani CF, Gaipa G, Lussana F *et al.* Sleeping Beauty-engineered CAR T cells achieve antileukemic activity without severe toxicities. *J. Clin. Invest*. 2020; 130, 6021–6033.

45. Sauter CS, Senechal B, Riviere I *et al.* CD19 CAR T cells following autologous transplantation in poor-risk relapsed and refractory B-cell non-Hodgkin lymphoma. *Blood* 2019; 134, 626–635.

46. Kochenderfer JN, Dudley ME, Feldman SA *et al.* B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* 2012; 119, 2709–2720.

47. Rossi J, Paczkowski P, Shen YW *et al.* Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. *Blood* 2018; 132, 804–814.

48. Brudno JN, Lam N, Vanasse D *et al.* Safety and feasibility of anti-CD19 CAR T cells with fully human binding domains in patients with B-cell lymphoma. *Nat. Med*. 2020; 26, 270–280.

49. Ritchie DS, Neeson PJ, Khot A *et al.* Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. *Mol. Ther*. 2013; 21, 2122–2129.

50. Siegler E, Li S, Kim YJ, Wang P. Designed Ankyrin Repeat Proteins as Her2 Targeting Domains in Chimeric Antigen Receptor-Engineered T Cells. *Hum. Gene. Ther*. 2017; 28, 726–736.

51. Han X, Cinay GE, Zhao Y, Guo Y, Zhang X, Wang P. Adnectin-Based Design of Chimeric Antigen Receptor for T Cell Engineering. *Mol. Ther*. 2017; 25, 2466–2476.

52. Zajc CU, Dobersberger M, Schaffner I, Mlynek G, Puhringer D, Salzer B, *et al.* A conformation-specific ON-switch for controlling CAR T cells with an orally available drug. *Proc. Natl Acad. Sci. USA*. 2020;117,14926–14935.

53. Salzer B, Schueller CM, Zajc CU, Peters T, Schoeber MA, Kovacic B, *et al.* Engineering AvidCARs for combinatorial antigen recognition and reversible control of CAR function. *Nat Commun*. 2020; 11, 4166.

54. Walsh ST, Cheng H, Bryson JW, Roder H, DeGrado WF. Solution structure and dynamics of a de novo designed three-helix bundle protein. *Proc. Natl Acad. Sci. USA*. 1999; 96, 5486–5491.

DOI: 10.18609/ioi.2022.003

55. Qin H, Edwards JP, Zaritskaya L *et al.* Chimeric Antigen Receptors Incorporating D Domains Targeting CD123 Direct Potent Mono- and Bi-specific Antitumor Activity of T Cells. *Mol Ther*. 2019; 27, 1262–1274.

56. Carpenter RO, Evbuomwan MO, Pittaluga S *et al.* B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. *Clin. Cancer. Res*. 2013; 19, 2048–60.

57. Shah N, Chari A, Scott E, Mezzi K, Usmani SZ. B-cell maturation antigen (BCMA) in multiple myeloma: rationale for targeting and current therapeutic approaches. *Leukemia* 2020; 34, 985–1005.

58. Novak AJ, Darce JR, Arendt BK, Harder B, Henderson K, Kindsvogel W, *et al.* Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival. *Blood* 2004; 103, 689–694.

59. Sanchez E, Gillespie A, Tang G *et al.* Soluble B-Cell Maturation Antigen Mediates Tumor-Induced Immune Deficiency in Multiple Myeloma. *Clin. Cancer Res*. 2016; 22, 3383–3397.

60. Sanchez E, Tanenbaum EJ, Patil S *et al.* The clinical significance of B-cell maturation antigen as a therapeutic target and biomarker. *Expert Rev. Mol. Diagn*. 2018; 18, 319–329.

61. Ghermezi M, Li M, Vardanyan S *et al.* Serum B-cell maturation antigen: a novel biomarker to predict outcomes for multiple myeloma patients. *Haematologica*. 2017; 102, 785–795.

62. Dalla Palma B, Marchica V, Catarozzo MT, Giuliani N, Accardi F. Monoclonal and Bispecific Anti-BCMA Antibodies in Multiple Myeloma. *J. Clin. Med*. 2020; 9, 3022.

63. Lonial S, Lee HC, Badros A *et al.* Belantamab mafodotin for relapsed or refractory multiple myeloma (DREAMM-2): a two-arm, randomised, open-label, phase 2 study. *Lancet Oncol*. 2020; 21, 207–221.

64. Lonial S, Lee HC, Badros A *et al.* Pivotal DREAMM-2 study: Single-agent belantamab mafodotin (GSK2857916) in patients with relapsed/refractory multiple myeloma (RRMM) refractory to proteasome inhibitors (PIs), immunomodulatory agents, and refractory and/or intolerant to anti-CD38 monoclonal antibodies (mAbs). Poster presented at: ASCO Annual meeting. Alexandria, VA. May 29–31 2020.

65. Yu B, Jiang T, Liu D. BCMA-targeted immunotherapy for multiple myeloma. *J. Hematol. Oncol*. 2020; 13, 125.

66. Sha F, Salzman G, Gupta A, Koide S. Monobodies and other synthetic binding proteins for expanding protein science. *Protein Sci*. 2017; 26, 910–24.

67. Zhu Y, Alonso DO, Maki K *et al.* Ultrafast folding of alpha3D: a de novo designed three-helix bundle protein. *Proc Natl Acad Sci USA*. 2003; 100, 15486–15491.

68. Buonato JM, Edwards J, Zaritskaya L *et al.* Design and Demonstration of Potent in vitro and in vivo Activity for CART ddBCMA , a BCMA Targeted CAR T Cell Therapy Incorporating a Non scFv Binding Domain. Poster presented at: *ASGCT Virtual Meeting*. May 11–14 2021

69. Frigault MJ, Rosenblatt J, Raje N *et al.* Phase 1 Study of CART-Ddbcma, a CAR-T Therapy Utilizing a Novel Synthetic Binding Domain for the Treatment of Subjects with Relapsed and /or Refractory Multiple Myeloma. Poster presented at: *ASH 63rd Annual Meeting*, Atlanta, GA. 14 Dec 2021.

70. Van Oekelen O, Aleman A, Upadhyaya B *et al.* Neurocognitive and hypokinetic movement disorder with features of parkinsonism after BCMA-targeting CAR-T cell therapy. *Nat. Med*. 2021; 27, 2099–2103.

AFFILIATIONS

Anand Rotte,
Employee of Arcellx

Christopher Heery
Employee of Arcellx

Bradford Gliner,
Employee of Arcellx

David Tice,
Employee of Arcellx

David M Hilbert,~
Employee of Arcellx

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *All authors are employees of Arcellx and hold stocks in the company. The authors declare that they have no other conflicts of interest.*

Funding declaration: *Other than regular emplyment, the authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Nov 30 2021;* ***Revised manuscript received:*** *Jan 12 2022;* ***Publication date:*** *Jan 24 2022.*

DOI: 10.18609/ioi.2022.003

IMMUNO-ONCOLOGY INSIGHTS

ANTICIPATING IMMUNO-ONCOLOGY MODALITY/ PLATFORM DEVELOPMENT TRENDS FOR 2022

SPOTLIGHT

INTERVIEW

Realizing the potential of NK cell therapy

ALVIN SHIH joined Catamaran as President and CEO in February 2021. He is an experienced physician and biopharma executive with a track record of building and leading companies that develop therapeutics addressing significant unmet medical needs. Most recently, Alvin was CEO of Disarm Therapeutics, a private neuroscience company that was acquired by Eli Lilly in 2020. Prior to Disarm, Alvin was CEO of Enzyvant Therapeutics, a company developing cell/tissue-based regenerative medicines for rare diseases. He previously held senior leadership roles at Retrophin and Pfizer, where he was the Chief Operating Officer of the Pfizer Rare Disease Research Unit. Alvin began his career as a strategy consultant at McKinsey & Co. and LEK Consulting. He holds an MD from the University of Alabama, an MBA from the Kellogg School of Management, and a BA from Vanderbilt University. Alvin completed his residency training in internal medicine at the Massachusetts General Hospital.

Immuno-Oncology Insights 2022; 3(1), 1–5

DOI: 10.18609/ioi.2022.001

Q What are you working on right now?

AS: We are working on building a company that is differentiated from the rest of the field in cell therapy, and that has a real chance of providing a transformative therapy for patients with solid tumors.

Q How would you sum up the story of NK cell therapy development to date?

AS: We know that cell therapy in general has come a long way in a short period of time and has been quite transformative for patients with hematologic malignancies in particular.

However, it's been a difficult transition to apply the learnings from hematologic malignancies to solid tumors. That is the next frontier that needs to be addressed for cell therapy to reach its full potential in treating tumors.

The other attractive feature of our platform is that NK cells play into the movement from autologous to allogeneic therapies. Autologous therapies (such as currently approved forms of CAR-T) are very useful but have been limited in uptake because of the logistical and technical challenges. If we can provide an allogeneic or off-the-shelf therapy, it will increase the reach of cell therapy in general.

Our engineered CAR-NK cells are allogeneic and have potent anti-tumor activity. From what we know about the biology and the early clinical experience, using NK cells may result in a cell therapy with a better safety profile than autologous CAR-T, at least with regard to severe outcomes like cytokine release syndrome (CRS), neurotoxicity, and graft vs. host disease (GvHD). It is a great chassis to be building on for this next generation of allogeneic therapies.

Q There are great expectations for further advancement in the field as we enter a new year, particularly in terms of applications in the solid tumor realm - what specifically is fuelling this optimism, for you?

AS: I am optimistic because of the great progress we've seen at Catamaran over a very short period of time, as well as progress in the cell therapy field overall. Cell therapy in general has been a robust area for clinical exploration as well as investment, so I am optimistic that the technologies being developed right now are going to increase the armamentarium of approaches that doctors have to hand.

One of the things that has been nice to see is the early but growing body of clinical data on CAR-NKs. The initial data coming out of the MD Anderson experience has certainly fueled optimism around the potential for NKs to be transformative in cancer therapy.

"We know that cell therapy in general has come a long way in a short period of time and has been quite transformative for patients with hematologic malignancies in particular."

DOI: 10.18609/ioi.2022.001

Q Tell us more about Catamaran's own platform & approach. What differentiates it in this increasingly busy space?

> "The initial data coming out of the MD Anderson experience has certainly fueled optimism around the potential for NKs to be transformative in cancer therapy."

AS: We have two dimensions on which we differentiate – our strategy and our technology.

On the strategic side, we are targeting solid tumors by first intent, which is different to most companies out there who are looking to go first into hematologic malignancies and then into solid tumors. We hope that the approaches targeting hematologic malignancies work, but we think there is something to be said for aiming for solid tumors where the greatest degree of unmet need lies. We know that about 80% of cancer diagnoses are in solid tumors, so it is important that those patients have some therapeutic options.

On the technology side, our means of engineering is different to most others in the space. Many companies use viral vectors to make genetic edits to their cells, whether they be autologous or allogeneic. Our approach is to use a transposon-based system to edit NK cells. This allows us to insert larger payloads than a typical viral vector would allow, and also enables us to design and manufacture these cells in a more efficient and scalable way. The larger payload opportunity means we can get more creative in the way we think about editing these cells. For instance, we are able to design in synthetic receptors that help our CAR-NK cells evade the immunosuppressive tumor microenvironment. Being able to survive – or even thrive – in the harsh tumor microenvironment will be an important strategy for addressing solid tumors.

Q What would you single out as they key challenges facing NK cell therapy developers such as yourselves moving forward & can you share some details of your plans to address them?

AS: One challenge that often gets raised is manufacturing. It does not matter how elegant your cell construct is if you cannot make it and get it into the hands of doctors who treat patients.

To manage this, we have invested very early on in building our own internal process development and manufacturing group, so we have ownership of the process and can more effectively manage the external vendors and collaborators we are working with. That is an important piece.

Another potential challenge is on the regulatory front. Regulations are constantly changing and especially with some of the recent reports of cell and gene therapies being placed on clinical hold by the FDA, it is quite possible that regulators will want tighter controls on cell therapies in the early stages of development moving forward. The mitigation strategy there is to have early and frequent engagement with regulators. We have certainly initiated that - we

have a scheduled interaction with the FDA in the early part of 2022 that will help inform the development pathway for our programs.

Q What are the likely next steps for the NK cell therapy field – for instance, in the combination therapy setting?

AS: We always think about possible combination approaches, and where a therapy like ours can be best applied. It's unclear what combinations will make sense, but we have the privilege of watching the rest of the field to see what sort of data emerges from approaches like antibodies, antibody drug conjugates, small molecules, and other cell therapies.

Each modality is going to play a role and add therapeutic options. I think combinations may ultimately extend the magnitude and durability of the clinical effects of individual therapies. It will be interesting to see if there is some combination of cell therapies that would make sense with each other. This will be enabled by an allogeneic approach making cell therapies more accessible and allowing a greater degree of experimentation with combination approaches.

Q Can you sum up some chief goals & priorities both for Catamaran over the next few years?

AS: It is going to be a really busy time ahead of us as we try to move our programs closer to the clinic in the most efficient way. We are prosecuting two programs in parallel. Our first program is targeting HER2 expressing solid tumors, specifically breast and gastric cancer. Our second program is targeting CD70 expressing tumors, such as renal cell carcinoma. Our priority over the next few years will be trying to get those moved along as quickly as possible so that we can get into the clinic and demonstrate that our platform has potential.

My own goal is to build the company to enable us to do that. Part of that is building the organization and hiring the best people who will allow us to move these programs forward and deliver therapies to patients.

AFFILIATION

Alvin Shih
President and CEO, Catamaran Bio

"I think combinations may ultimately extend the magnitude and durability of the clinical effects of individual therapies."

 DOI: 10.18609/ioi.2022.001

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author declare that they have no conflicts of interest.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *Dec 9 2021;* ***Publication date:*** *Jan 24 2022.*