Revitope Oncology, Inc.
www.revitope.com

Revitope—third-generation T cell engager immunotherapies

Revitope is developing conditionally activated, T cell–engaging, bispecific antibodies containing dual tumor-specific antigens and split T cell–targeting paratopes that are unique in their high tolerability. The company is now focused on advancing its therapies to deliver safer and more efficacious therapies especially in solid cancer settings.

Revitope Oncology, Inc. is a privately owned company in Cambridge, MA, that focuses on the development of next-generation T cell engager immunotherapies for a variety of solid cancer indications. Revitope's platform is a suite of proprietary and modular bispecific antibodies designed to deliver improved therapeutic efficacy and safety through built-in control mechanisms that enable exquisite tumor-specificity. The company has several cancer programs in preclinical development and expects to have its lead program in the clinic by early 2023.

Over the past decade, T cell engager immunotherapies have proven efficacious in treating various cancers but their clinical utility has been limited by extreme toxicity of mainly two types: 'on-target, off-tumor' immune activation leading to serious immune related adverse events in healthy tissues and cytokine release syndrome (CRS).

Revitope's Precision Guided Antibody Tumor Engager (*Precision*GATE) platform technology addresses these issues through a unique dual antigen design, the *Two*GATE system, engineered to precisely target cancer cells and elicit a focused and powerful immune response that eradicates the tumor, leaving healthy tissue unharmed. *Two*GATE consists of dual antigen T cell engagers that require the presence of two different tumor-specific antigens expressed on the same tumor cell to become active, significantly minimizing toxicity risk and widening the therapeutic window for treatment of previously inaccessible cancers (Fig. 1).

Revitope is seeking to identify potential partners interested in tailoring *Two*GATE for their own selected target antigens co-expressed on cancer cells. Revitope's internal programs include REV-400, the company's lead program in solid cancer. REV-400 is designed to bind two antigens co-expressed on lung and on other solid tumor types that have been notoriously difficult to target with traditional immunotherapy approaches. The company's pipeline includes several other solid cancer programs in development either in house or in partnership with collaborators.

"Bispecific antibodies that can simultaneously target a cancer cell and a T cell are unique in their ability to direct an immune response directly on tumor cells without the need for tumor-specific T cells or neoantigen presentation on cancer cells," said Steve Arkinstall, CEO of Revitope. "But, while this immuno-oncology modality has certainly revolutionized treatment options for patients with certain cancers, its broader application has been hampered by high toxicities and on-target effects on healthy cells. At Revitope, with our team of creative and highly engaged scientists and industry veterans, we have engineered next-generation therapeutic T cell engagers that are active only when they bind two different antigens expressed on the same tumor cell. Together with several other safety features, this makes our bispecific antibodies unique in their specificity and their ability to minimize toxicities. We are now focused on advancing our T cell engager therapies to deliver safer and more efficacious therapies."

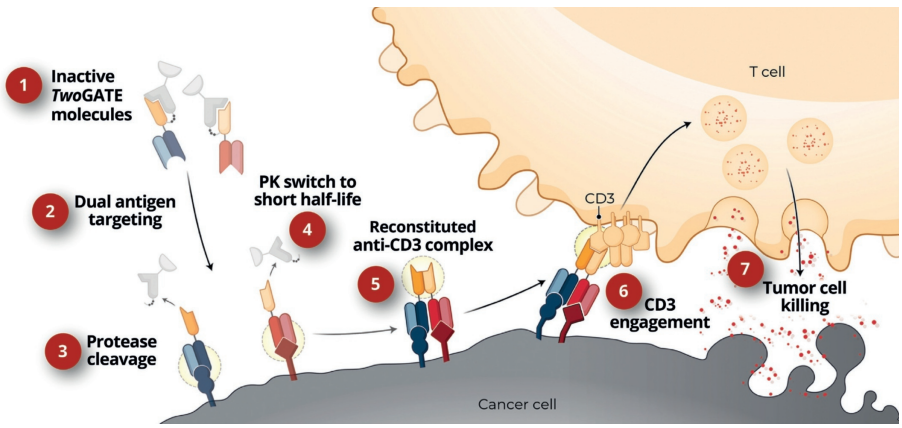

Fig. 1 | Revitope's *Two*GATE technology. *Two*GATE (Two component Guided Antibody Tumor Engager) consists of two bispecific antibodies each targeting a distinct tumor antigen and forming an active anti-CD3 complex only when bound to the surface of a cancer cell. Each of the CD3-targeting split paratopes are associated with a stabilizing domain and can only be activated by tumor-specific proteases. In addition, the bispecific antibodies are engineered to contain a PK switch—a half-life extension domain that is lost upon proteolytic activation within the tumor microenvironment—that ensures any active *Two*GATE molecule not bound to a tumor cell is rapidly cleared from the body. The *Two*GATE technology dramatically minimizes toxicity risk and widens the therapeutic window of treatment for previously considered inaccessible cancers.

Evolving T cell engager immunotherapies

Because of their ability to engage two targets simultaneously, bispecific antibodies amplify the therapeutic possibilities of monoclonal antibodies (mAbs), and a rapidly expanding industry pipeline attests to the recognized potential of such applications. In cancer immunotherapy, the ability of bispecific antibodies to form 'immunological synapses' between tumor cells and immune effector cells such as T cells, affords the possibility to precisely target an immunological response to a particular cancer cell.

First-generation bispecific antibodies were designed to target a single tumor-specific antigen with one of its arms and a CD3 subunit of the T cell receptor with the other arm. While efficacious, these have been marred by 'on-target, off-tumor' toxicities due to residual expression of tumor-specific antigens on healthy tissues. These toxicities are caused by broad immune cell activation and could trigger cytokine release syndrome (CRS) and immune-related adverse events.

To avoid these side effects, second-generation T cell engager bispecific antibodies were developed containing masked paratopes—the small antigen binding sites on an antibody—targeting single tumor-specific antigens, and a CD3 subunit of the T cell receptor. Paratope masking refers to the engineering of prodrug versions of the bispecific antibody in which the paratopes are protected by a peptide that can only be released in the presence of a tumor-specific protease. The goal of this approach is to reduce on-target, off-tumor–related toxicities by minimizing the chances of healthy tissues expressing the tumor-specific antigens from triggering an immune response.

However, low-level protease activities present outside the tumor microenvironment could limit the broad application of these second generation

T cell engagers due to 'de-masking' of their highly active anti-CD3 binding paratope, which may trigger unwanted activation in healthy tissues. This is akin to a firearm safety switch which is inadvertently turned off. Revitope's focus has been on further improving the T cell engager technology to more precisely target cancer cells and direct the immunological responses exclusively on tumors.

*Two*GATE—the sum of many parts

Building on the lessons learned from the development of the first-and second-generation T cell engagers, Revitope used its deep knowledge of the mechanism of action of immune-based drugs as well as its expertise in protein engineering to design its two component *Two*GATE T cell engager platform. This system incorporates a two-step T cell activation process that allows for highly precise tumor targeting and a controlled immune activation. Using the firearm analogy, Revitope opted to apply a safety switch and to remove the ammunition until required.

Revitope's third-generation T cell engager consists of two bispecific antibodies, each of which incorporates a binding module for a tumor antigen as well as half of the domain required to bind a CD3 subunit of the T cell receptor. This idea of splitting the anti-CD3 paratope ensures that each of the two drug components is completely inactive until both bind simultaneously to target antigens on the same tumor cell. On the cell's surface, both halves of the anti-CD3 paratope associate to form a complex able to bind and activate T cells. Importantly, formation of the active anti-CD3 complex cannot occur when free in solution, such as tissue fluid or blood. A critical safety feature linked to the split anti-CD3 paratope is that each of the bispecific antibodies is designed to bind a distinct tumor antigen, making dual-antigen binding an absolute requirement to trigger T cell activation. Careful selection of antigens essentially expands the universe of cancer antigens available for specific targeting of solid tumors.

The quantum improvement in this design is the exponential increase in specificity by requiring the presence of two different tumor-specific antigens on the same target tumor cell, a threshold that minimizes and in most instances completely eliminates the probability of a healthy cell binding both bispecifics and reconstituting a CD3 binding complex that could engage a T cell and cause an adverse effect (Fig. 2).

In addition to the dual antigen requirement, Revitope incorporated two further safety layers in its *Two*GATE system. First, it engineered a 'stabilizing domain' associated with the split anti-CD3 paratope, similar to the masking domain approach previously tested in some second-generation T cell engagers. Just as was the case with those bispecifics, this ensures that the active anti-CD3 complex can only be formed in the presence of tumor-specific protease enzymes, substantially limiting the possibility of healthy cells engaging T cells. A further safety layer consists of a pharmacokinetic (PK) switch engineered into Revitope's T cell engagers, ensuring that any activated *Two*GATE molecules not bound to a tumor cell are rapidly cleared from the body. This PK switch consists of a half-life extension domain linked to the stabilizing domain but lost upon proteolytic cleavage within the tumor microenvironment.

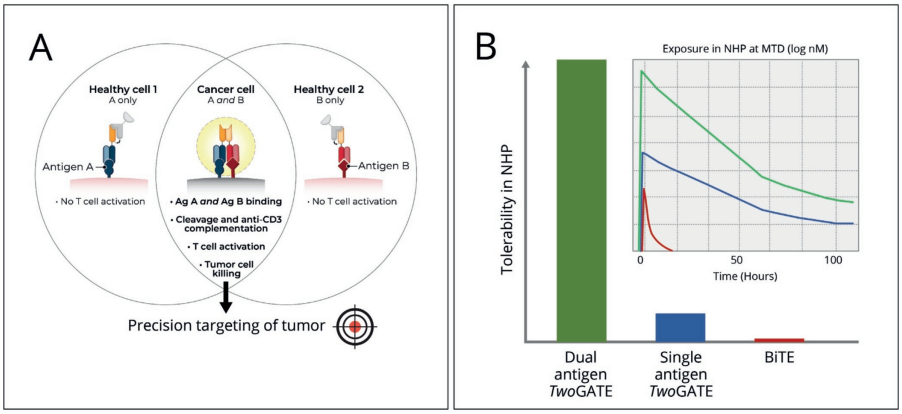

Fig. 2 | Revitope's *Two*GATE T cell engager activation requires both dual antigen binding and 'protease' cleavage to provide the highest tolerability among bispecific antibody–based T cell engager immunotherapies. (**A**) Venn diagram depiction of *Two*GATE activation and lysis of tumor cells co-expressing target antigens A and B. Healthy cells expressing either antigen A or B fail to trigger T cell activation so avoiding toxicity. (**B**) In vivo experiments in a non-human primate safety study reveal the safety advantage of dual antigen targeting *Two*GATE molecules over single antigen targeting either by *Two*GATE or a classic BiTE molecule. BiTE, bispecific T cell engager; NHP, non-human primate.

"Our dual antigen/split paratope combination is a truly differentiated way of delivering greater selectivity and expands the target space beyond that available to other T cell engagers," said Werner Meier, Revitope's CSO. "Selecting the right combination of tumor-specific antigens is obviously key to our approach, and our data show that the improved outcomes are due to both the novel dual antigen activation step and the stacking of several safety features, including proteolytic release of the stabilizing domain and a PK switch. Using this drug design we have identified many cancer antigens that hold promise for a much higher degree of tumor specific targeting than single antigens will ever be able to provide."

Revitope has already shown a high expression potential of the bispecific antibody components in CHO cells (yields >2 g/L), exceptional freeze-thaw and high temperature stabilities, and drug product solubilities of ≥20 mg/mL. Revitope will soon initiate CMC and IND-enabling studies with its lead program, and plans to enter clinical studies in early 2023. Despite their apparent complexity, *Two*GATE molecules behave like monoclonal antibodies in their biophysical properties.

A unique partnering opportunity

With its platform reaching maturity following validation in vitro and in vivo, Revitope is starting to build a network of biopharmaceutical partnerships to advance its novel T cell engager immunotherapies across a range of indications. In particular, Revitope is interested in identifying co-development partners interested in collaborating on advancing other programs based on the company's *Two*GATE platform. This modular platform is ideally suited for partnering as it can be rapidly tailored and leverages proven well profiled antibody components that will reduce development risk and time to clinic.

In 2020, the company entered its first two strategic partnerships. The first was a research collaboration and license deal with Shanghai-based Junshi Biosciences to generate up to five dual antigen T cell engagers for cancer worth up to $800m. The second was a research collaboration with US-based Janssen Biotech to evaluate the *Two*GATE T cell engager platform and explore the possibility of a broader collaboration and license agreement.

"We believe Revitope offers excellent opportunities for partners who can leverage our proprietary *Two*GATE technology to generate safer and more effective therapies addressing a broader range of targets in both solid and hematological cancers," said Mark Clement, CFO and CBO of Revitope. "We are the only player in the field of conditionally activated bispecific T cell engagers to offer robust and potent T cell engagers with significantly greater tolerability in vitro and in vivo, including in non-human primates, than existing first- and second-generation bispecific platforms, and we believe this opens an untapped universe of target space. Our vision is to advance this unique platform into the clinic ourselves and in partnership with others interested in bringing the promise of immunotherapy to as many cancer patients as possible."

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> Mark Clement, CFO & CBO, Revitope

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