CASE STUDIES
Antibody-antigen complex structures

ANTIBODY-ANTIGEN
STRUCTURES

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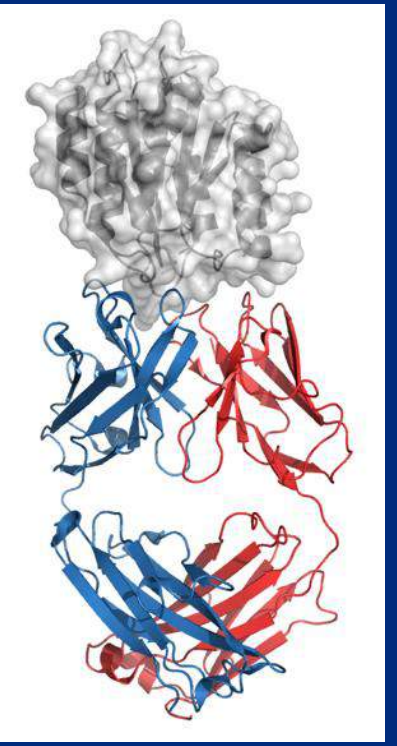
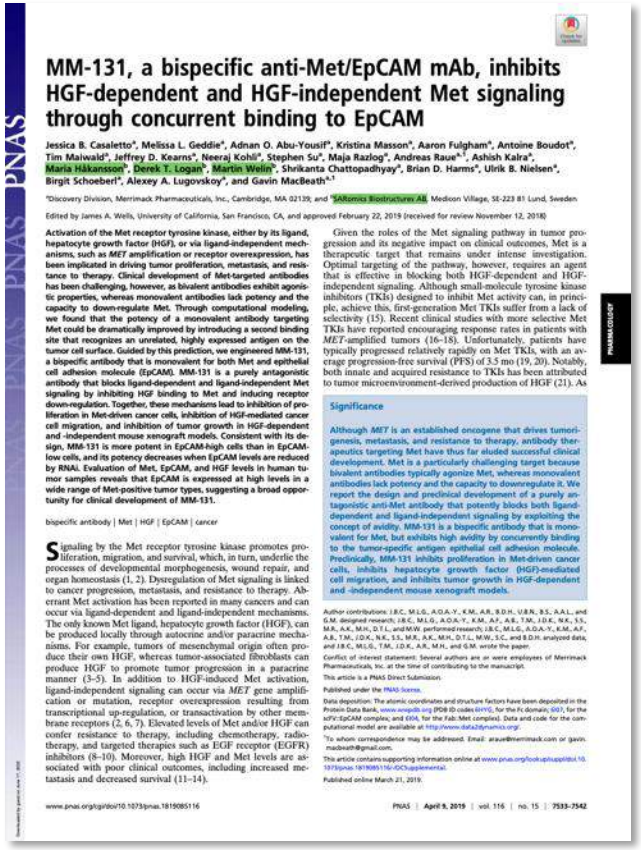


MM-131 – Antigen Structures Case Study



MM-131, a bispecific anti-Met/EpCAM mAb, inhibits HGF-dependent and HGF-independent Met signaling through concurrent binding to EpCAM

Jessica B. Casaletto[a], Melissa L. Geddie[a], Adnan O. Abu-Yousif[a], Kristina Masson[a], Aaron Fulgham[a], Antoine Boudot[a], Tim Maiwald[a], Jeffrey D. Kearns[a], Neeraj Kohli[a], Stephen Su[a], Maja Razlog[a], Andreas Raue[a,1], Ashish Kalra[a], Maria Håkansson[b], Derek T. Logan[b], Martin Welin[b], Shrikanta Chattopadhyay[a], Brian D. Harms[a], Ulrik B. Nielsen[a], Birgit Schoeberl[a], Alexey A. Lugovskoy[a], and Gavin MacBeath[a,1]

[a]Discovery Division, Merrimack Pharmaceuticals, Inc., Cambridge, MA 02139; and [b]SARomics Biostructures AB, Medicon Village, SE-223 81 Lund, Sweden

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Activation of the Met receptor tyrosine kinase, either by its ligand, hepatocyte growth factor (HGF), or via ligand-independent mechanisms, such as *MET* amplification or receptor overexpression, has been implicated in driving tumor proliferation, metastasis, and resistance to therapy. Clinical development of Met-targeted antibodies has been challenging, however, as bivalent antibodies exhibit agonistic properties, whereas monovalent antibodies lack potency and the capacity to down-regulate Met. Through computational modeling, we found that the potency of a monovalent antibody targeting Met could be dramatically improved by introducing a second binding site that recognizes an unrelated, highly expressed antigen on the tumor cell surface. Guided by this prediction, we engineered MM-131, a bispecific antibody that is monovalent for both Met and epithelial cell adhesion molecule (EpCAM). MM-131 is a purely antagonistic antibody that blocks ligand-dependent and ligand-independent Met signaling by inhibiting HGF binding to Met and inducing receptor down-regulation. Together, these mechanisms lead to inhibition of proliferation in Met-driven cancer cells, inhibition of HGF-mediated cancer cell migration, and inhibition of tumor growth in HGF-dependent and -independent mouse xenograft models. Consistent with its design, MM-131 is more potent in EpCAM-high cells than in EpCAM-low cells, and its potency decreases when EpCAM levels are reduced by RNAi. Evaluation of Met, EpCAM, and HGF levels in human tumor samples reveals that EpCAM is expressed at high levels in a wide range of Met-positive tumor types, suggesting a broad opportunity for clinical development of MM-131.

bispecific antibody | Met | HGF | EpCAM | cancer

Signaling by the Met receptor tyrosine kinase promotes proliferation, migration, and survival, which, in turn, underlie the processes of developmental morphogenesis, wound repair, and organ homeostasis (1, 2). Dysregulation of Met signaling is linked to cancer progression, metastasis, and resistance to therapy. Aberrant Met activation has been reported in many cancers and can occur via ligand-dependent and ligand-independent mechanisms. The only known Met ligand, hepatocyte growth factor (HGF), can be produced locally through autocrine and/or paracrine mechanisms. For example, tumors of mesenchymal origin often produce their own HGF, whereas tumor-associated fibroblasts can produce HGF to promote tumor progression in a paracrine manner (3–5). In addition to HGF-induced Met activation, ligand-independent signaling can occur via *MET* gene amplification or mutation, receptor overexpression resulting from transcriptional up-regulation, or transactivation by other membrane receptors (2, 6, 7). Elevated levels of Met and/or HGF can confer resistance to therapy, including chemotherapy, radiotherapy, and targeted therapies such as EGF receptor (EGFR) inhibitors (8–10). Moreover, high HGF and Met levels are associated with poor clinical outcomes, including increased metastasis and decreased survival (11–14).

Given the roles of the Met signaling pathway in tumor progression and its negative impact on clinical outcomes, Met is a therapeutic target that remains under intense investigation. Optimal targeting of the pathway, however, requires an agent that is effective in blocking both HGF-dependent and HGF-independent signaling. Although small-molecule tyrosine kinase inhibitors (TKIs) designed to inhibit Met activity can, in principle, achieve this, first-generation Met TKIs suffer from a lack of selectivity (15). Recent clinical studies with more selective Met TKIs have reported encouraging response rates in patients with *MET*-amplified tumors (16–18). Unfortunately, patients have typically progressed relatively rapidly on Met TKIs, with an average progression-free survival (PFS) of 3.5 mo (19, 20). Notably, both innate and acquired resistance to TKIs has been attributed to tumor microenvironment-derived production of HGF (21). As

Significance

Although *MET* is an established oncogene that drives tumorigenesis, metastasis, and resistance to therapy, antibody therapeutics targeting Met have thus far eluded successful clinical development. Met is a particularly challenging target because bivalent antibodies typically agonize Met, whereas monovalent antibodies lack potency and the capacity to downregulate it. We report the design and preclinical development of a purely antagonistic anti-Met antibody that potently blocks both ligand-dependent and ligand-independent signaling by exploiting the concept of avidity. MM-131 is a bispecific antibody that is monovalent for Met, but exhibits high avidity by concurrently binding to the tumor-specific antigen epithelial cell adhesion molecule. Preclinically, MM-131 inhibits proliferation in Met-driven cancer cells, inhibits hepatocyte growth factor (HGF)-mediated cell migration, and inhibits tumor growth in HGF-dependent and -independent mouse xenograft models.

Author contributions: J.B.C., M.L.G., A.O.A.-Y., K.M., A.R., B.D.H., U.B.N., B.S., A.A.L., and G.M. designed research; J.B.C., M.L.G., A.O.A.-Y., K.M., A.F., A.B., T.M., J.D.K., N.K., S.S., M.R., A.K., M.H., D.T.L., and M.W. performed research; J.B.C., M.L.G., A.O.A.-Y., K.M., A.F., A.B., T.M., J.D.K., N.K., S.S., M.R., A.K., M.H., D.T.L., M.W., S.C., and B.D.H. analyzed data; and J.B.C., M.L.G., T.M., J.D.K., A.R., M.H., and G.M. wrote the paper.

Conflict of interest statement: Several authors are or were employees of Merrimack Pharmaceuticals, Inc. at the time of contributing to the manuscript.

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Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.wwpdb.org (PDB ID codes 6HYG, for the Fc domain; 6I07, for the scFv:EpCAM complex; and 6I04, for the Fab:Met complex). Data and code for the computational model are available at http://www.data2dynamics.org/.

[1]To whom correspondence may be addressed. Email: araue@merrimack.com or gavin.macbeath@gmail.com.

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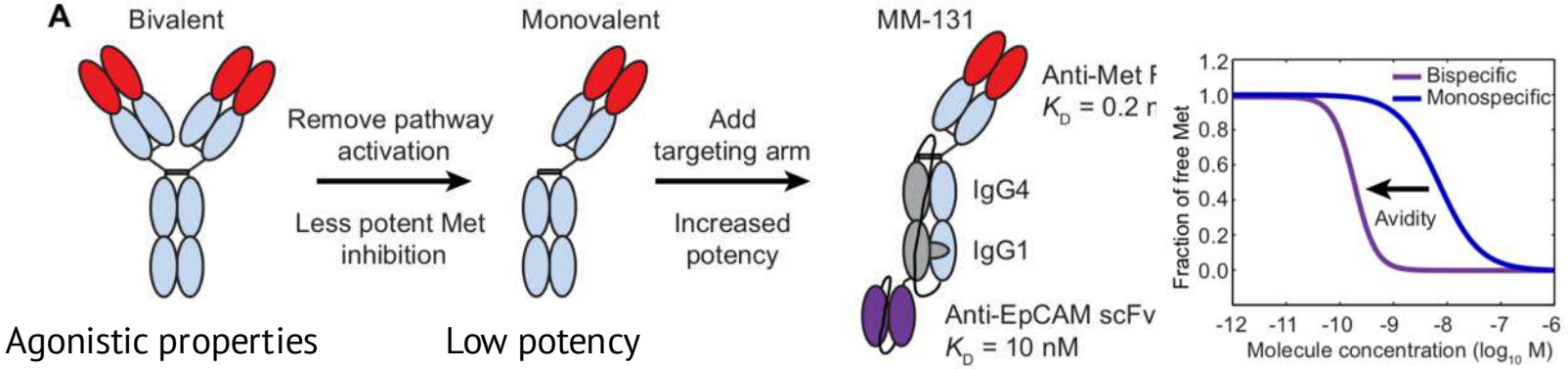
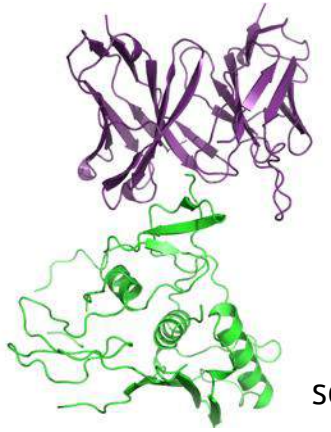


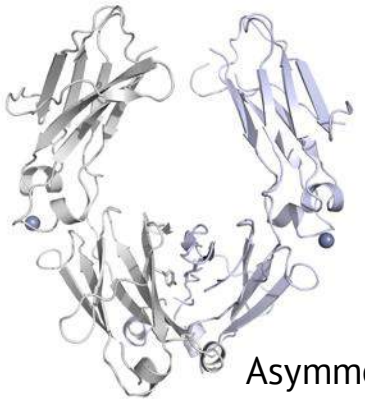
Agonistic properties

Low potency

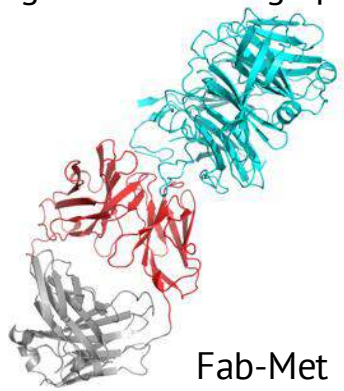
Antagonistic with high potency



scFv-EpCAM



Asymmetric Fc



Fab-Met

PDB codes: 6I07, 6HYG, 6I04

Casaletto *et al.*, 2019, *PNAS*, 116, 7533-7542.

Bispecific Antibody Mediating PD-L1–Dependent CD28 Co-stimulation on T Cells for Enhanced Tumor Control

CANCER IMMUNOLOGY RESEARCH | RESEARCH ARTICLE

NI-3201 Is a Bispecific Antibody Mediating PD-L1–Dependent CD28 Co-stimulation on T Cells for Enhanced Tumor Control

Sara Majocchi[1], Pauline Lloveras[1], Lise Nouveau[1], Margaux Legrand[1], Alizee Viandier[1], Pauline Malinge[1], Maud Charreton[1], Cecile Raymond[1], Emily A. Pace[2], Bjorn L. Millard[2], L. Anders Svensson[3], Vinardas Kelpšas[3], Nadia Anceriz[1], Susana Salgado-Pires[1], Bruno Daubeuf[1], Giovanni Magistrelli[1], Franck Gueneau[1], Valéry Moine[1], Krzysztof Masternak[1], Limin Shang[1], Nicolas Fischer[1], and Walter G. Ferlin[1]

ABSTRACT

Despite advances in cancer immunotherapy, such as targeting the PD-1/PD-L1 axis, a substantial number of patients harbor tumors that are resistant or relapse. Selective engagement of T-cell co-stimulatory molecules with bispecific antibodies may offer novel therapeutic options by enhancing signal 1–driven activation occurring via T-cell receptor engagement. In this study, we report the development and preclinical characterization of NI-3201, a PD-L1×CD28 bispecific antibody generated on the κλ-body platform that was designed to promote T-cell activity and antitumor function through a dual mechanism of action. We confirmed that NI-3201 blocks the PD-L1/PD-1 immune checkpoint pathway and conditionally provides T-cell co-stimulation via CD28 (signal 2) when engaging PD-L1$^+$ tumors or immune cells. In systems with signal 1–primed T cells, NI-3201 enhanced potent effector functionality: *in vitro* through antigen-specific recall assays with cytomegalovirus-specific T cells and *in vivo* by inducing tumor regression and immunologic memory in tumor-associated antigen–expressing MC38 syngeneic mouse models. When T-cell engagers were used to provide synthetic signal 1, the combination with NI-3201 resulted in synergistic T cell–dependent cytotoxicity and potent antitumor activity in two humanized mouse tumor models. Nonhuman primate safety assessments showed favorable tolerability and pharmacokinetics at pharmacologically active doses. Quantitative systems pharmacology modeling predicted that NI-3201 exposure results in antitumor activity in patients, but this remains to be investigated. Overall, this study suggests that by combining PD-L1 blockade with safe and effective CD28 co-stimulation, NI-3201 has the potential to improve cancer immunotherapy outcomes, and the clinical development of NI-3201 for PD-L1$^+$ solid tumors is planned.

Introduction

The advent of immunotherapy has led to a remarkable revolution in cancer treatment over the past decade (1, 2). Immunotherapy leverages the power of the immune system to eradicate cancer cells. One of the most notable breakthroughs was the targeting of the PD-L1/PD-1 immune checkpoint (IC) axis (3). PD-L1 expressed on tumor cells binds to PD-1 on T cells, leading to the inhibition of immune responses via dephosphorylation of T-cell receptor (TCR)/CD28 proximal signaling molecules (4, 5). mAbs that block ICs, termed IC inhibitors (ICI), unleash antitumor T-cell responses, leading to tumor regression and long-term survival in a subset of patients across a variety of cancer types (6, 7).

Despite the undeniable success of ICIs, a substantial proportion of patients do not experience clinical benefit (8). The reasons for this variable response are multifaceted and are not fully understood. Tumor heterogeneity, an immunosuppressive microenvironment, and intrinsic resistance mechanisms within cancer cells can contribute to treatment failure (8). Neutralizing antidrug antibodies (ADA) may also negatively affect treatment (9). This highlights the need for novel therapeutic strategies that expand the responder population and address the limitations of current immunotherapies.

Agonist antibodies designed to target co-stimulatory pathways in T cells have emerged as promising candidates for immunotherapy (10). Such antibodies mimic the natural activation signals received by T cells through co-stimulatory molecules, thereby enhancing their effector function and proliferation. CD28 is one of the primary co-stimulatory receptors on T cells, and upon engagement with its ligands CD80/CD86 on antigen-presenting cells, CD28 delivers a co-stimulatory signal that synergizes with the TCR signal to promote T-cell cytokine production, survival, differentiation, and expansion (11).

Several CD28-specific mAbs have been proposed for therapeutic targeting of CD28. A fraction, termed superagonist (SA) antibodies, induces full activation of primary resting T cells even in the absence of TCR ligation (signal 1). The first-in-human study of the high-affinity (1.88 nmol/L) SA anti-CD28 TGN1412 resulted in catastrophic outcomes with cytokine release syndrome and multiorgan failure in all administered healthy volunteers (12).

Although the outcomes associated with TGN1412 cast doubt about the use of CD28 agonists, the receptor itself remained a potentially promising target for immunotherapy. Emerging strategies have focused on developing safer and more targeted approaches to exploit CD28 in cancer treatment (13). One promising strategy involves the use of bispecific antibodies (bsAb) that simultaneously engage CD28 and a tumor-associated antigen (TAA), directing T-cell activation specifically toward cancer cells while minimizing

[1]Light Chain Bioscience – Novimmune SA, Geneva, Switzerland. [2]Residual Dynamics, Camano Island, Washington. [3]AAtomics Biostructures AB, Lund, Sweden.

Corresponding Author: Walter G. Ferlin, Light Chain Bioscience – Novimmune SA, 15 Chemin du Pré-Fleuri, Plan-les-Ouates, Geneva 1228, Switzerland. E-mail: walter.ferlin@lightchainbio.com

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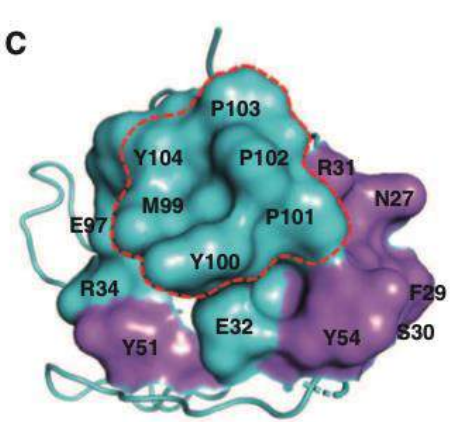
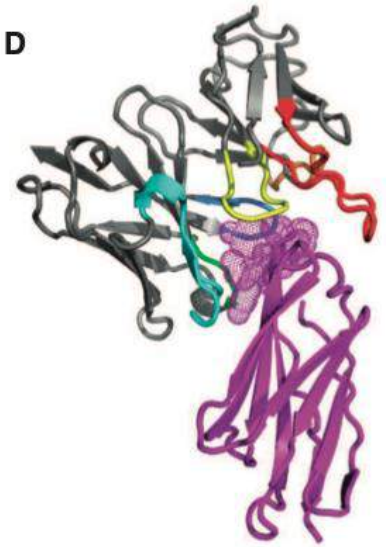
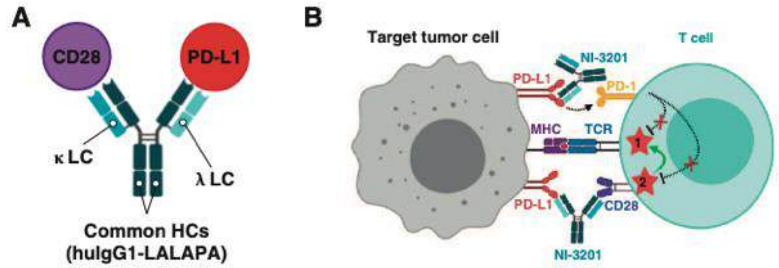


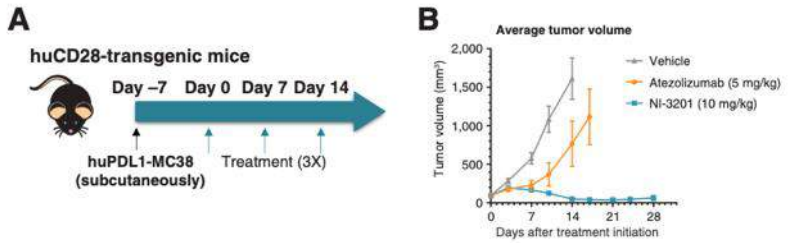


Crystal structure of the anti-CD28 κ-arm Fab complexed to the extracellular domain of human CD28 (pink, with the MYPPPY motif highlighted by a cloud) and the Fab CDRH1–3 in green, cyan, and blue, and CDRL1–3 in red, orange, and yellow, respectively (PDB code: 8S6Z).

Residues of the Fab epitope that are unique to CD28 are shown in purple, whereas residues that are shared between CD28 and CTLA-4 are shown in aqua blue.



NI-3201 mediates CD28 co-stimulation upon PD-L1 blockade.



NI-3201 shows strong single-agent antitumor activity in immunocompetent huCD28 mice.

Majocchi et al., 2024, Cancer Immunol Res, 0298.

Structural analysis of light chain-driven bispecific antibodies targeting CD47 and PD-L1

MABS
2024, VOL. 16, NO. 1, 2362432
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REPORT

OPEN ACCESS

Structural analysis of light chain-driven bispecific antibodies targeting CD47 and PD-L1

Pauline Malinge[a]*, Xavier Chauchet[a]*, Jérémie Bourguignon[a], Nicolas Bosson[a], Sébastien Calloud[a], Tereza Bautzova[a], Marie Borlet[a], Mette Laursen[b], Vinardas Kelpsas[b], Nadia Rose[b], Franck Gueneau[a], Ulla Ravn[a], Giovanni Magistrelli[a], and Nicolas Fischer[a]

[a]Light Chain Bioscience - Novimmune SA, Plan-les-Ouates, Switzerland; [b]SARomics Biostructures AB, Lund, Sweden

ABSTRACT

In contrast to natural antibodies that rely mainly on the heavy chain to establish contacts with their cognate antigen, we have developed a bispecific antibody format in which the light chain (LC) drives antigen binding and specificity. To better understand epitope-paratope interactions in this context, we determined the X-ray crystallographic structures of an antigen binding fragment (Fab) in complex with human CD47 and another Fab in complex with human PD-L1. These Fabs contain a κ-LC and a λ-LC, respectively, which are paired with an identical heavy chain (HC). The structural analysis of these complexes revealed the dominant contribution of the LCs to antigen binding, but also that the common HC provides some contacts in both CD47 and PD-L1 Fab complexes. The anti-CD47 Fab was affinity optimized by diversifying complementary-determining regions of the LC followed by phage display selections. Using homology modeling, the contributions of the amino acid modification to the affinity increase were analyzed. Our results demonstrate that, despite a less prominent role in natural antibodies, the LC can mediate high affinity binding to different antigens and neutralize their biological function. Importantly, Fabs containing a common variable heavy (VH) domain enable the generation of bispecific antibodies retaining a truly native structure, maximizing their therapeutic potential.

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KEYWORDS
Bispecific; light chain; CD47; PD-L1; structure; X-ray crystallography

Introduction

Antibodies are a key component of the mammalian adaptive immune system used to fight pathogens. Beyond their biological importance, the exquisite specificity of antibodies has led to their widespread use as research tools, diagnostics, and therapeutic agents. Indeed, since the description of hybridoma fusion to generate the first mouse-derived monoclonal antibodies in 1975, the evolution of antibody generation and engineering technologies have led to the establishment of antibodies as one of the most successful and fast-growing classes of therapeutic modalities. [1–3]

A natural IgG antibody is composed of four polypeptides, two copies of a heavy chain (HC) and two copies of a light chain (LC). The variable regions of the HC and LC each contain three hypervariable loops, named complementarity-determining regions (CDRs), that contact the antigen and thus determine the specificity and affinity of the antibody. Antibody-antigen interfaces can vary significantly in shape and area (from 300 to 900 Å^2), depending on the size and nature of the antigen. As the structure of more than a thousand intact IgGs or IgG fragments has been solved (www.rcsb.org), the structural features of CDRs and their contributions to antigen binding are well understood. [4–7] The CDR3 of HC and LC (H3 and L3, respectively) are located at the center of the paratope and invariably contribute to interaction with the antigen. In general, the binding is skewed toward the HC with CDRs H3, H2 and L3 providing most of the contact points. [8,9] This is also due to the dominant role of H3, which is the most diverse CDR in length and sequence and is always involved in antigen contact. [10,11] The high diversity of CDR H3 is created during B cell development by two recombination events linking V, D, and J regions, as well as nucleotide additions and deletions at the junctions of these segments. [12,13]

In the past two decades, many approaches and technologies to generate bispecific antibodies (bsAbs) capable of engaging two different antigens, or two different epitopes on the same antigen, have been developed. [14–16] In some cases, the VH and VL of both antibodies are combined into a single molecule by using linkers (e.g., single-chain variable fragment (scFv)) or reshaping interfaces to favor the correct chain pairing and thus maintain the original antibody specificities. [17] To simplify correct chain pairing, the use of antibodies that bear a common and invariable LC has emerged as a widespread strategy. In this case the binding is even more skewed toward the HC, as the LC is not diversified. [18–20]

We previously described a different approach to generate native bsAbs by co-expressing a common HC and two

CONTACT Nicolas Fischer Nicolas.Fischer@lightchainbio.com Light Chain Bioscience - Novimmune SA, Chemin du Pré-Fleuri 15, Plan-les-Ouates 1228, Switzerland

*Contributed equally to this work.

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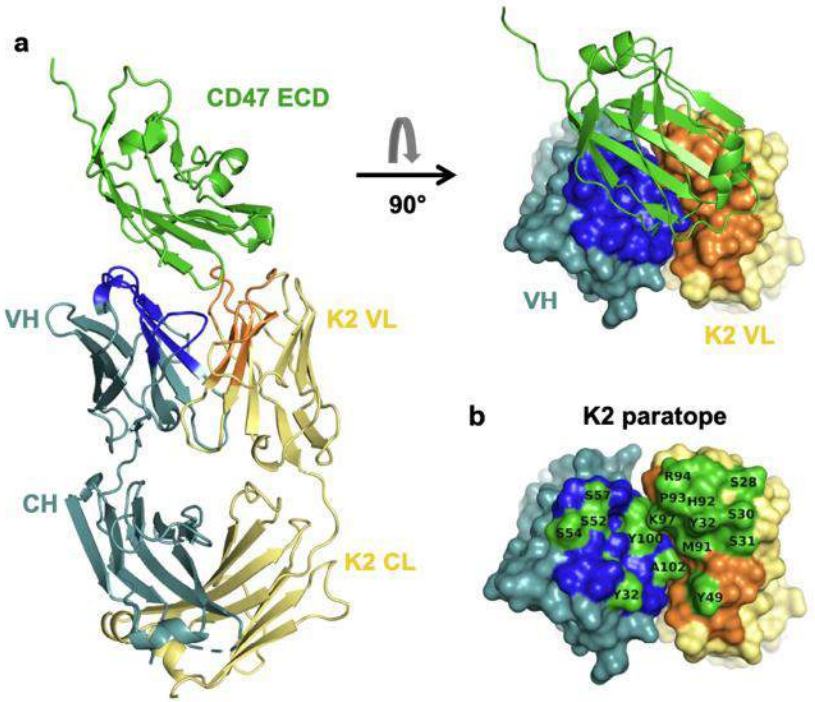
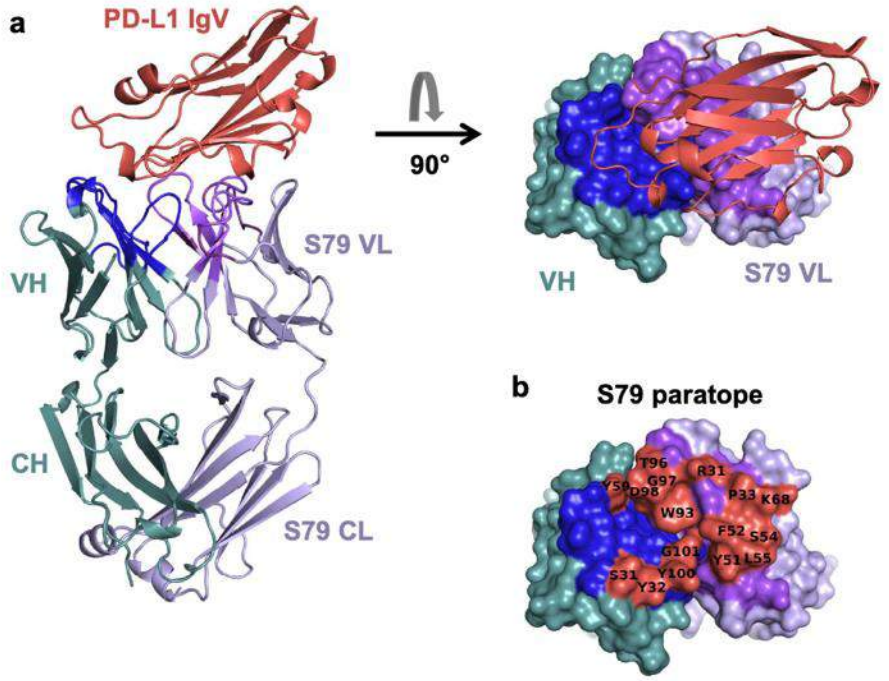


Crystal structure of IgG1 with κ-light chain bound to CD47 (PDB code: 8RP8).



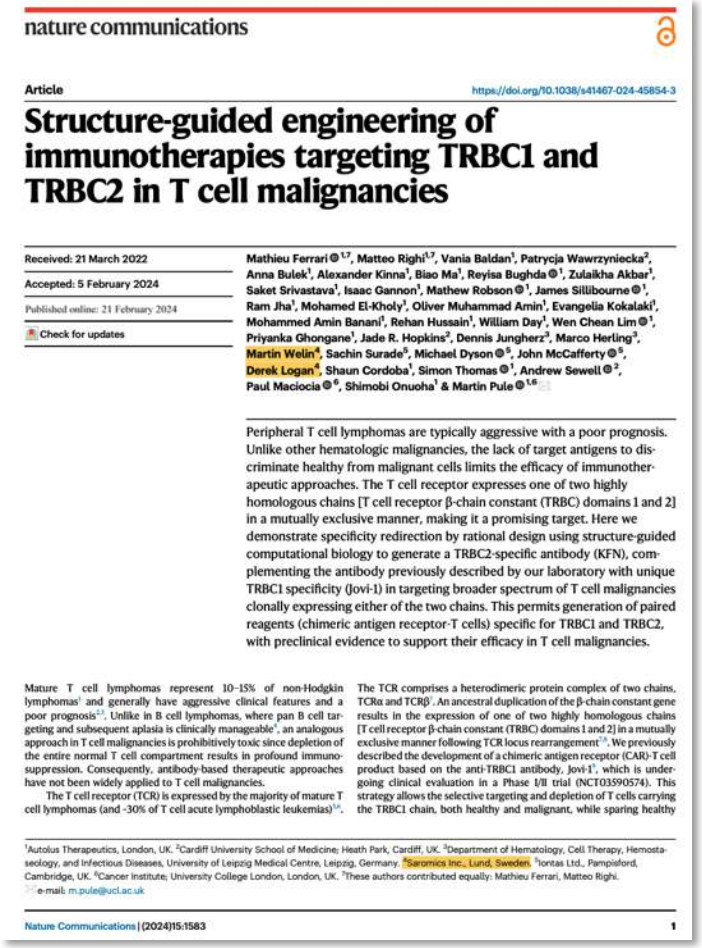
Crystal structure of IgG4 with λ-light chain bound to PD-L1 (PDB code: 8RPB).

Malinge et al., 2024, mAbs, 16, 2362432.

Structure-guided engineering of immunotherapies targeting TRBC1 and TRBC2 in T cell malignancies

Client project: Jovi-1 Fab in complex with T cell receptor β-chain constant (TRBC) domains

Collaboration with Autolus Therapeutics, London, UK



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Structure-guided engineering of immunotherapies targeting TRBC1 and TRBC2 in T cell malignancies

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Check for updates

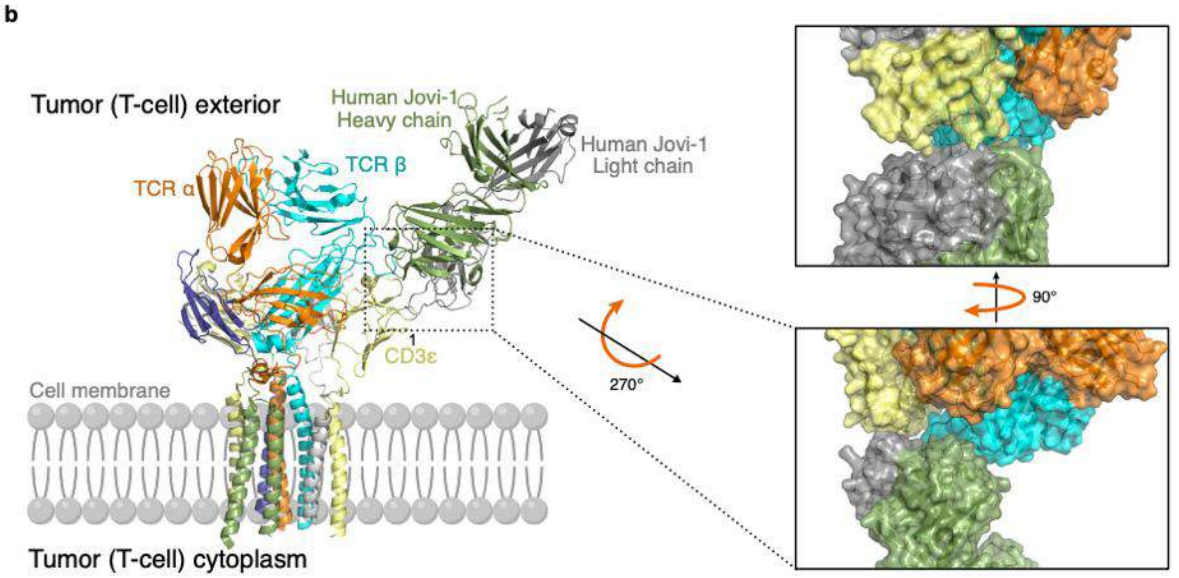
Mathieu Ferrari[1,7], Matteo Righi[1,7], Vania Baldan[1], Patrycja Wawrzyniecka[2], Anna Bulek[1], Alexander Kinna[1], Biao Ma[1], Reyisa Bughda[1], Zulaikha Akbar[1], Saket Srivastava[1], Isaac Gannon[1], Mathew Robson[1], James Sillibourne[1], Ram Jha[1], Mohamed El-Kholy[1], Oliver Muhammad Amin[1], Evangelia Kokalaki[1], Mohammed Amin Banani[1], Rehan Hussain[1], William Day[1], Wen Chean Lim[1], Priyanka Ghongane[1], Jade R. Hopkins[3], Dennis Jungherz[3], Marco Herling[3], Martin Welin[4], Sachin Surade[5], Michael Dyson[5], John McCafferty[5], Derek Logan[4], Shaun Cordoba[1], Simon Thomas[1], Andrew Sewell[2], Paul Maciocia[6], Shimobi Onuoha[1] & Martin Pule[1,6]

Peripheral T cell lymphomas are typically aggressive with a poor prognosis. Unlike other hematologic malignancies, the lack of target antigens to discriminate healthy from malignant cells limits the efficacy of immunotherapeutic approaches. The T cell receptor expresses one of two highly homologous chains [T cell receptor β-chain constant (TRBC) domains 1 and 2] in a mutually exclusive manner, making it a promising target. Here we demonstrate specificity redirection by rational design using structure-guided computational biology to generate a TRBC2-specific antibody (KFN), complementing the antibody previously described by our laboratory with unique TRBC1 specificity (Jovi-1) in targeting broader spectrum of T cell malignancies clonally expressing either of the two chains. This permits generation of paired reagents (chimeric antigen receptor-T cells) specific for TRBC1 and TRBC2, with preclinical evidence to support their efficacy in T cell malignancies.

Mature T cell lymphomas represent 10–15% of non-Hodgkin lymphomas[1] and generally have aggressive clinical features and a poor prognosis[2,3]. Unlike in B cell lymphomas, where pan B cell targeting and subsequent aplasia is clinically manageable[4], an analogous approach in T cell malignancies is prohibitively toxic since depletion of the entire normal T cell compartment results in profound immunosuppression. Consequently, antibody-based therapeutic approaches have not been widely applied to T cell malignancies.

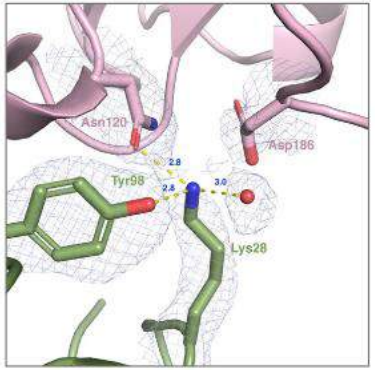
The T cell receptor (TCR) is expressed by the majority of mature T cell lymphomas (and ~30% of T cell acute lymphoblastic leukemias)[5,6]. The TCR comprises a heterodimeric protein complex of two chains, TCRα and TCRβ[7]. An ancestral duplication of the β-chain constant gene results in the expression of one of two highly homologous chains [T cell receptor β-chain constant (TRBC) domains 1 and 2] in a mutually exclusive manner following TCR locus rearrangement[7,8]. We previously described the development of a chimeric antigen receptor (CAR)-T cell product based on the anti-TRBC1 antibody, Jovi-1[9], which is undergoing clinical evaluation in a Phase I/II trial (NCT03590574). This strategy allows the selective targeting and depletion of T cells carrying the TRBC1 chain, both healthy and malignant, while sparing healthy

[1]Autolus Therapeutics, London, UK. [2]Cardiff University School of Medicine; Heath Park, Cardiff, UK. [3]Department of Hematology, Cell Therapy, Hemostaseology, and Infectious Diseases, University of Leipzig Medical Centre, Leipzig, Germany. [4]Saromics Inc., Lund, Sweden. [5]Iontas Ltd., Pampisford, Cambridge, UK. [6]Cancer Institute; University College London, London, UK. [7]These authors contributed equally: Mathieu Ferrari, Matteo Righi.
e-mail: m.pule@ucl.ac.uk



Superimposition of Human Jovi-1 Fab-TCR complex on TCR CD3 complex structure showing how specificity for TRBC is mediated in the context of the CD3 sheath.

PDB codes: 7AMP, 7AMQ, 7AMR and 7AMS



- To facilitate a viable treatment strategy, TRBC2-targeting agents were sought
- Specificity to TRBC2 was achieved by structure-guided engineering of the antibody targeting TRBC1
- The mutant antibody, showed a 3-log decrease in TRBC1 affinity and a 15-fold increase in TRBC2 affinity compared with the parent antibody

Ferrari et al., 2024, Nat Commun, 15,

Structure-based engineering of a novel CD3ε-targeting antibody for reduced polyreactivity

MABS
2023, VOL. 15, NO. 1, 2189974
https://doi.org/10.1080/19420862.2023.2189974

Taylor & Francis

REPORT

OPEN ACCESS

Structure-based engineering of a novel CD3ε-targeting antibody for reduced polyreactivity

Catherine Y Liu[a], Cory L Ahonen[a], Michael E Brown[a], Ling Zhou[a], Martin Welin[b], Eric M Krauland[a], Robert Pejchal[a], Paul F Widboom[a], and Michael B Battles[a]

[a]Adimab, LLC, NH, USA; [b]SARomics Biostructures AB, Lund, Sweden

ABSTRACT

Bispecific antibodies continue to represent a growth area for antibody therapeutics, with roughly a third of molecules in clinical development being T-cell engagers that use an anti-CD3 binding arm. CD3 antibodies possessing cross-reactivity with cynomolgus monkey typically recognize a highly electronegative linear epitope at the extreme N-terminus of CD3 epsilon (CD3ε). Such antibodies have high isoelectric points and display problematic polyreactivity (correlated with poor pharmacokinetics for monospecific antibodies). Using insights from the crystal structure of anti-Hu/Cy CD3 antibody ADI-26906 in complex with CD3ε and antibody engineering using a yeast-based platform, we have derived high-affinity CD3 antibody variants with very low polyreactivity and significantly improved biophysical developability. Comparison of these variants with CD3 antibodies in the clinic (as part of bi- or multi-specifics) shows that affinity for CD3 is correlated with polyreactivity. Our engineered CD3 antibodies break this correlation, forming a broad affinity range with no to low polyreactivity. Such antibodies will enable bispecifics with improved pharmacokinetic and safety profiles and suggest engineering solutions that will benefit the large and growing sector of T-cell engagers.

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Revised 21 February 2023
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KEYWORDS
Antibody; bispecifics; CD3; developability; polyreactivity; RTCC; structure; X-ray

Introduction

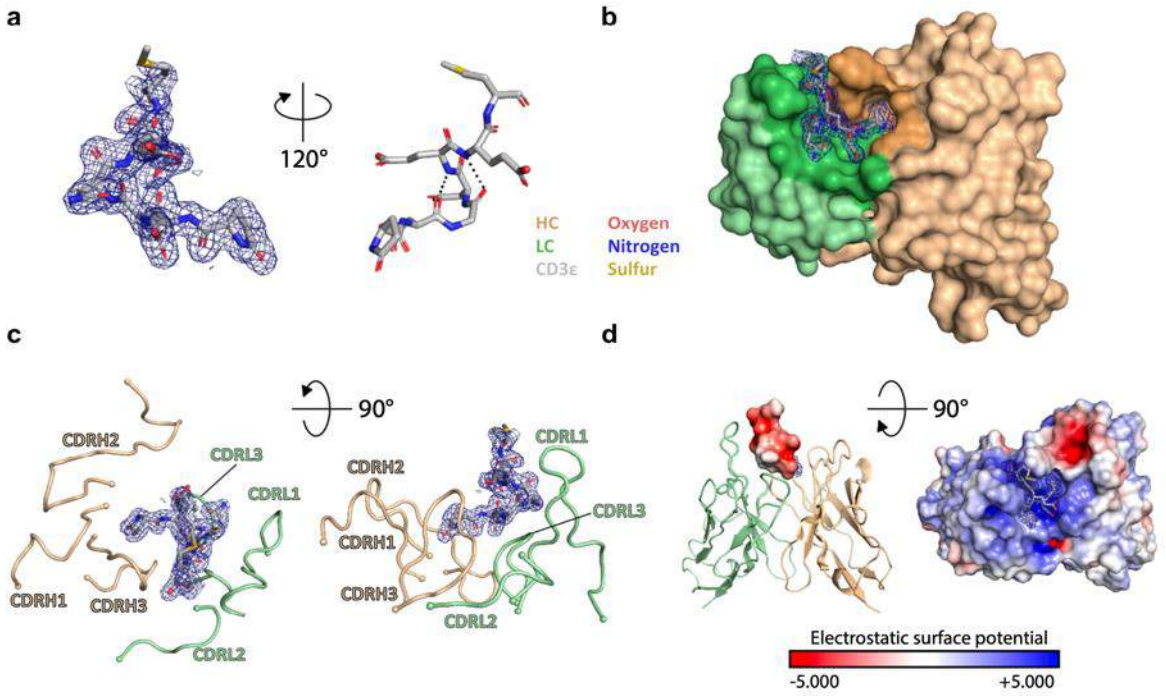
Immune-cell engaging bispecific antibodies are a promising class of therapeutics that have shown potential in treating both hematologic and solid tumor malignancies.[1] Simultaneously engaging cytotoxic T cells or natural killer cells and tumor cells via a tumor-associated antigen (TAA), these bispecific antibodies engage immune cells to kill cancerous cells that have evaded the immune system.[2,3] Starting with blinatumomab, the first bispecific T-cell engaging antibody to be approved by the US Food and Drug Administration (FDA) for patient treatment, many bispecific therapeutics are moving toward clinical use and some have more recently been approved in the US and European Union, include the T-cell engagers tebentafusp, teclistamab, and mosunetuzumab.[4–6]

T-cell stimulation is mediated by the T cell receptor (TCR)-CD3 complex with CD3 as the signaling component, where CD3 needs to be cross-linked to facilitate T-cell activation.[7] Stimulation leads to early activation markers CD69 and CD25 being transcriptionally upregulated on the T-cell surface.[8,9] These markers regulate the magnitude of the T cell proliferative response. Stimulation also causes the T cell to release pro-inflammatory cytokines such as IL-2, IFNγ, TNFα/β and others for Th1-biased cells. A growing body of literature suggests that CD3-targeting bispecific antibodies mimic the principles of kinetic segregation rooted in the mechanism of TCR/pMHC-mediated immunological synapse formation.[10,11] The resulting TCR signaling coupled with cross-linking of cytotoxic CD8+ T-cells to targets cells expressing the TAA arm of the bispecific molecule can redirect cytotoxic effects toward the targeted cells.[10–12]

For a bispecific antibody to be therapeutically effective, it must simultaneously engage the correct effector and target cells to elicit killing and have favorable pharmacokinetic (PK) properties. Numerous assays have been developed to assess developability concerns in antibodies during preclinical development. Such assays include baculovirus particle (BVP) and polyspecificity reagent (PSR) binding, as well as heparin sulfate chromatography, which assesses nonspecific binding, and affinity-capture self-interaction nanoparticle spectroscopy (AC-SINS), which measures propensity for antibody self-interaction.[13–20]

Historically, the terms polyspecificity and polyreactivity have been used interchangeably. Recently, there has been an effort to distinguish between the two such that polyspecificity refers to antibodies displaying "specific" and moderate off-target binding affinity to a discrete number of proteins that are not structurally or functionally related to the intended target antigen.[21] On the other hand, polyreactivity refers to an antibody's ability to bind nonspecifically to many unrelated proteins and lipids with weak affinity. These "sticky" interactions are thought to be encoded by excessive charge or hydrophobic content in the antibody variable fragment (Fv).[22] We believe our PSR assay identifies polyreactivity, as defined this way, and therefore adopt this terminology herein. Through the process of heteroligation, polyreactivity can enhance the

CONTACT Michael B Battles michael.battles@adimab.com Adimab, LLC, Lebanon, NH 03766, USA
Supplemental data for this article can be accessed online at https://doi.org/10.1080/19420862.2023.2189974.



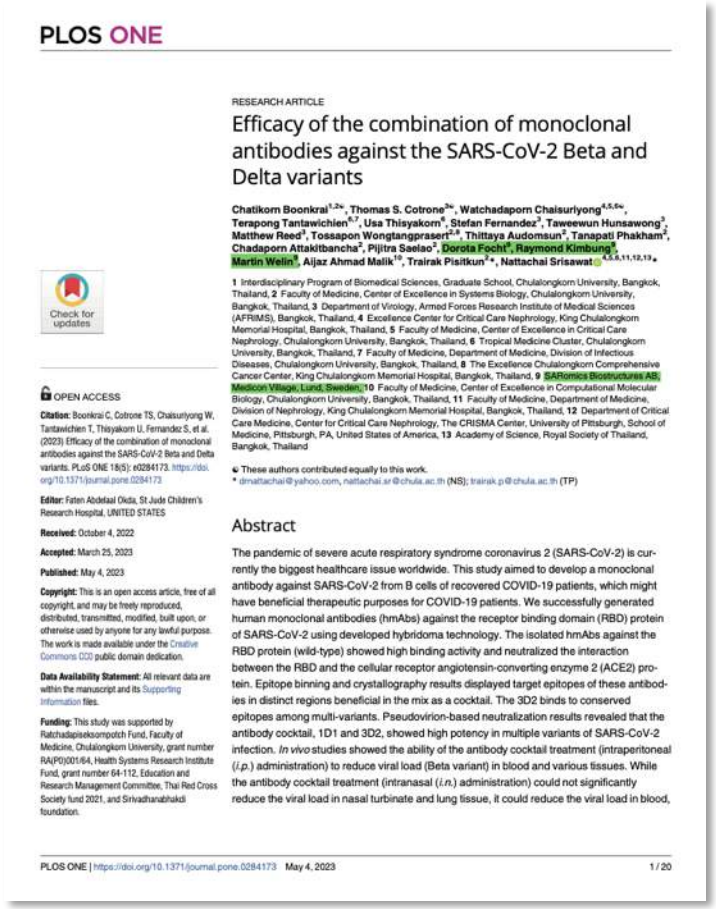
X-ray structure of ADI-26906 Fab in complex with N-terminal peptide from CD3ε

- CD3 antibodies possessing cross-reactivity with cynomolgus monkey typically recognize a highly electronegative linear epitope at the extreme N-terminus of CD3ε
- Using insights from the crystal structure of anti-Hu/Cy CD3 antibody ADI-26906 in complex with CD3ε and engineering, we have derived high-affinity CD3 antibody variants with very low polyreactivity and significantly improved biophysical developability.

PDB code: 8F0L

Liu et al., 2023, MAbs, 15, 2189974-2189974.

SARS-CoV-2 Fab complexes



PLOS ONE

RESEARCH ARTICLE

Efficacy of the combination of monoclonal antibodies against the SARS-CoV-2 Beta and Delta variants

Chatikorn Boonkrai[1,2☯], Thomas S. Cotrone[2☯], Watchadaporn Chaisuriyong[4,5,6☯], Terapong Tantawichien[6,7], Usa Thisyakorn[8], Stefan Fernandez[3], Taweewun Hunsawong[3], Matthew Reed[3], Tossapon Wongtangprasert[2,9], Thittaya Audomsun[2], Tanapati Phakham[2], Chadaporn Attakitbancha[2], Pijitra Saelao[2], Dorota Focht[9], Raymond Kimbung[9], Martin Welin[9], Aijaz Ahmad Malik[10], Trairak Pisitkun[2]*, Nattachai Srisawat[4,5,6,11,12,13]*

1 Interdisciplinary Program of Biomedical Sciences, Graduate School, Chulalongkorn University, Bangkok, Thailand, **2** Faculty of Medicine, Center of Excellence in Systems Biology, Chulalongkorn University, Bangkok, Thailand, **3** Department of Virology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand, **4** Excellence Center for Critical Care Nephrology, King Chulalongkorn Memorial Hospital, Bangkok, Thailand, **5** Faculty of Medicine, Center of Excellence in Critical Care Nephrology, Chulalongkorn University, Bangkok, Thailand, **6** Tropical Medicine Cluster, Chulalongkorn University, Bangkok, Thailand, **7** Faculty of Medicine, Department of Medicine, Division of Infectious Diseases, Chulalongkorn University, Bangkok, Thailand, **8** The Excellence Chulalongkorn Comprehensive Cancer Center, King Chulalongkorn Memorial Hospital, Bangkok, Thailand, **9** SARomics Biostructures AB, Medicon Village, Lund, Sweden, **10** Faculty of Medicine, Center of Excellence in Computational Molecular Biology, Chulalongkorn University, Bangkok, Thailand, **11** Faculty of Medicine, Department of Medicine, Division of Nephrology, King Chulalongkorn Memorial Hospital, Bangkok, Thailand, **12** Department of Critical Care Medicine, Center for Critical Care Nephrology, The CRISMA Center, University of Pittsburgh, School of Medicine, Pittsburgh, PA, United States of America, **13** Academy of Science, Royal Society of Thailand, Bangkok, Thailand

☯ These authors contributed equally to this work.
* drnattachai@yahoo.com, nattachai.sr@chula.ac.th (NS); trairak.p@chula.ac.th (TP)

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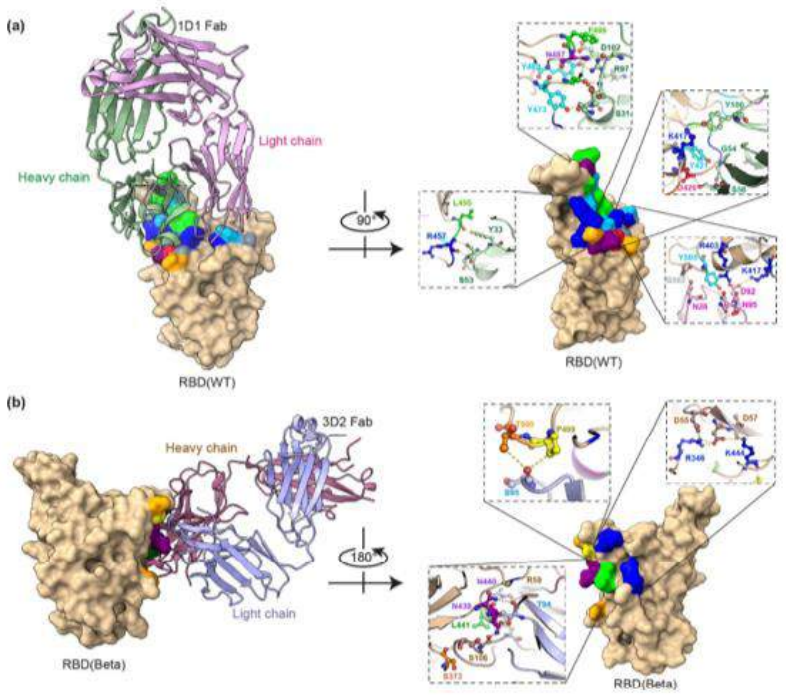
Abstract

The pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently the biggest healthcare issue worldwide. This study aimed to develop a monoclonal antibody against SARS-CoV-2 from B cells of recovered COVID-19 patients, which might have beneficial therapeutic purposes for COVID-19 patients. We successfully generated human monoclonal antibodies (hmAbs) against the receptor binding domain (RBD) protein of SARS-CoV-2 using developed hybridoma technology. The isolated hmAbs against the RBD protein (wild-type) showed high binding activity and neutralized the interaction between the RBD and the cellular receptor angiotensin-converting enzyme 2 (ACE2) protein. Epitope binning and crystallography results displayed target epitopes of these antibodies in distinct regions beneficial in the mix as a cocktail. The 3D2 binds to conserved epitopes among multi-variants. Pseudovirion-based neutralization results revealed that the antibody cocktail, 1D1 and 3D2, showed high potency in multiple variants of SARS-CoV-2 infection. *In vivo* studies showed the ability of the antibody cocktail treatment (intraperitoneal (*i.p.*) administration) to reduce viral load (Beta variant) in blood and various tissues. While the antibody cocktail treatment (intranasal (*i.n.*) administration) could not significantly reduce the viral load in nasal turbinate and lung tissue, it could reduce the viral load in blood,



Crystal structures of two Fab fragment complexes.

PDB code: 8BSE, 8BSF

Boonkrai et al., 2023, PLoS ONE, 18(5): e0284173.

Acknowledgments

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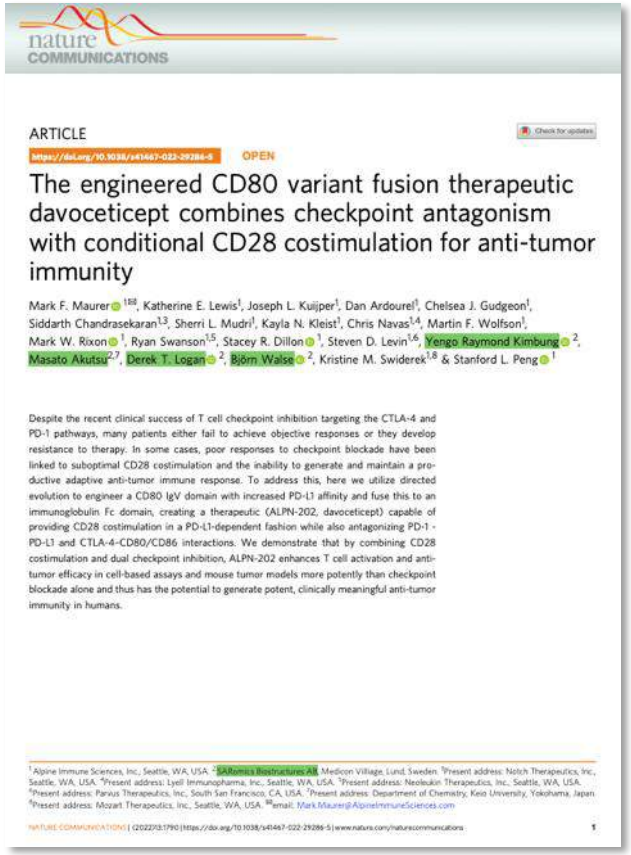
Davoceticept (ALPN-202) - An engineered CD80 variant fusion therapeutic



nature COMMUNICATIONS

ARTICLE

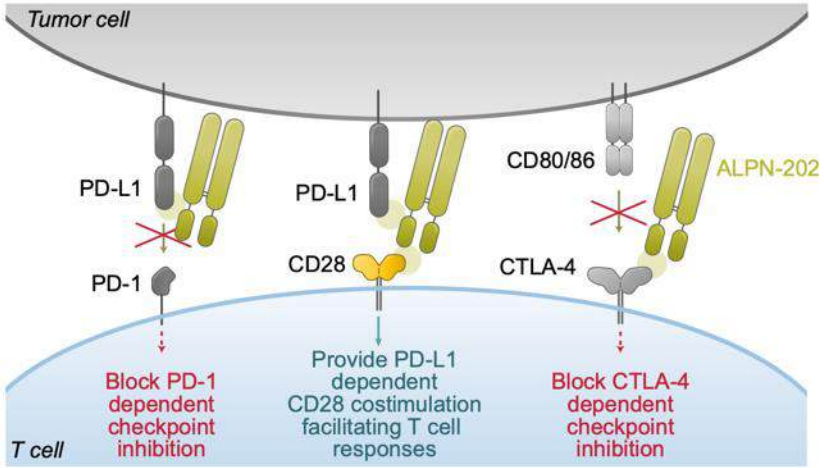
OPEN

The engineered CD80 variant fusion therapeutic davoceticept combines checkpoint antagonism with conditional CD28 costimulation for anti-tumor immunity

Mark F. Maurer, Katherine E. Lewis, Joseph L. Kuijper, Dan Ardourel, Chelsea J. Gudgeon, Siddarth Chandrasekaran, Sherri L. Mudri, Kayla N. Kleist, Chris Navas, Martin F. Wolfson, Mark W. Rixon, Ryan Swanson, Stacey R. Dillon, Steven D. Levin, Yengo Raymond Kimbung, Masato Akutsu, Derek T. Logan, Björn Walse, Kristine M. Swiderek & Stanford L. Peng

Despite the recent clinical success of T cell checkpoint inhibition targeting the CTLA-4 and PD-1 pathways, many patients either fail to achieve objective responses or they develop resistance to therapy. In some cases, poor responses to checkpoint blockade have been linked to suboptimal CD28 costimulation and the inability to generate and maintain a productive adaptive anti-tumor immune response. To address this, here we utilize directed evolution to engineer a CD80 IgV domain with increased PD-L1 affinity and fuse this to an immunoglobulin Fc domain, creating a therapeutic (ALPN-202, davoceticept) capable of providing CD28 costimulation in a PD-L1-dependent fashion while also antagonizing PD-1 - PD-L1 and CTLA-4-CD80/CD86 interactions. We demonstrate that by combining CD28 costimulation and dual checkpoint inhibition, ALPN-202 enhances T cell activation and anti-tumor efficacy in cell-based assays and mouse tumor models more potently than checkpoint blockade alone and thus has the potential to generate potent, clinically meaningful anti-tumor immunity in humans.

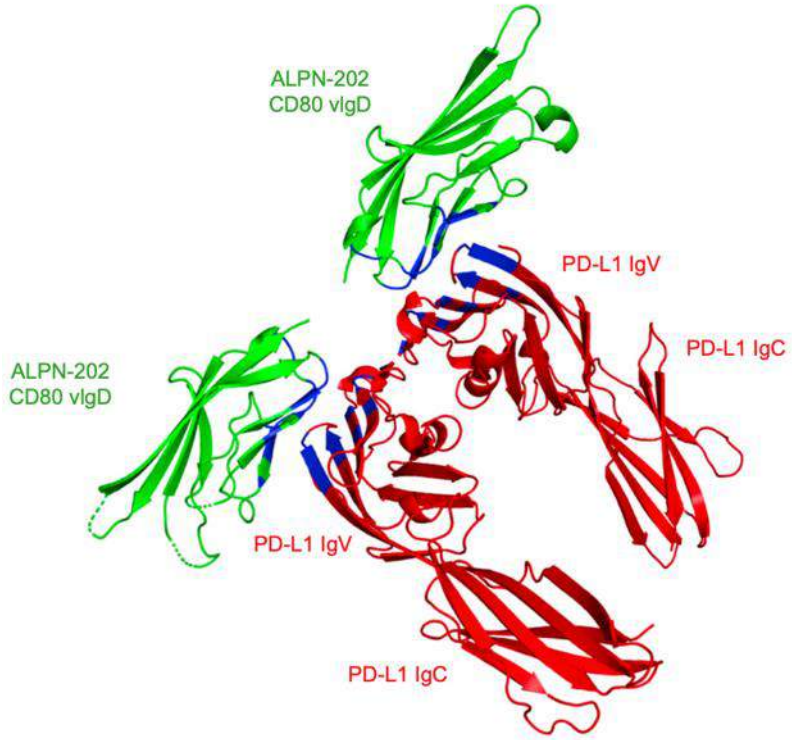
Published in Nature Communications!



The three mechanisms of action of ALPN-202:

- Blockade of PD-1–PD-L1 interaction
- PD-L1-dependent CD28 costimulation
- Blockade of CTLA-4–CD80/CD86 interactions.

PDB code: 7TPS



X-ray structure of ALPN-202 CD80 vIgD in complex with PD-L1

Maurer et al., 2022, Nat Comm, 13:1790.

Structural basis of activation and antagonism of receptor signaling mediated by interleukin-27

Cell Reports

CellPress
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Article

Structural basis of activation and antagonism of receptor signaling mediated by interleukin-27

Katarzyna Skladanowska,[1,2,14] Yehudi Bloch,[1,2,14] Jamie Strand,[3,9] Kerry F. White,[3,10] Jing Hua,[3,11] Daniel Aldridge,[4] Martin Welin,[5] Derek T. Logan,[5] Arne Soete,[6,7] Romain Merceron,[1,2,12] Casey Murphy,[1,2,13] Mathias Provost,[1,2] J. Fernando Bazan,[2,8] Christopher A. Hunter,[4] Jonathan A. Hill,[3,*] and Savvas N. Savvides[1,2,15,*]

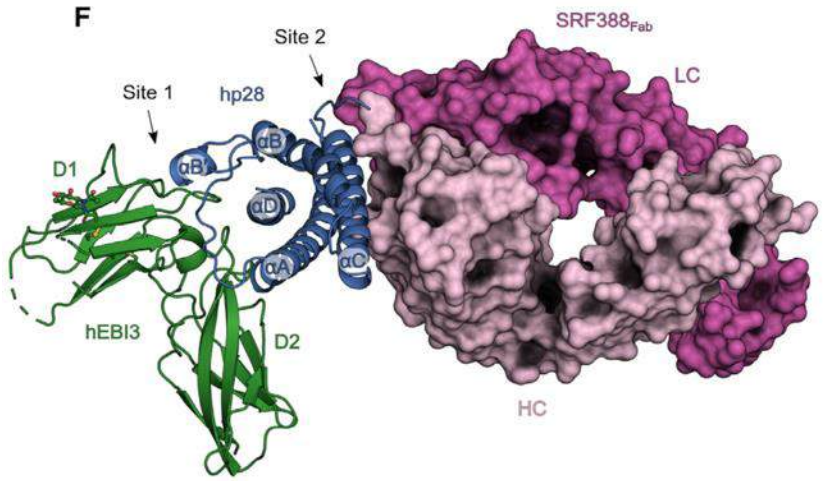
[1]Unit for Structural Biology, Department of Biochemistry and Microbiology Ghent University, Technologiepark 71, 9052 Ghent, Belgium
[2]Unit for Structural Biology, VIB-UGent Center for Inflammation Research, Technologiepark 71, 9052 Ghent, Belgium
[3]Surface Oncology, 50 Hampshire Street, Cambridge, MA 02139, USA
[4]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA
[5]SAPtomics Biostructures AB, Medicon Village, Scheelevägen 2, 223 63 Lund, Sweden
[6]Department of Biomedical Molecular Biology, Faculty of Science, Ghent University, Ghent, Belgium
[7]Data Mining and Modeling for Biomedicine, VIB-UGent Center for Inflammation Research, Ghent, Belgium
[8]b Bioconsulting, Stillwater, MN, USA
[9]Present address: NextPoint Therapeutics, 450 Kendall Street, Cambridge, MA 02142, USA
[10]Present address: Phenomic AI, 661 University Avenue, Toronto, ON M5G0B7, Canada
[11]Present address: Novartis Institute for Biomedical Research (NIBR), Cambridge, MA, USA
[12]Present address: Eurofins DiscoverX Products France (EDPF), Le Bois L'Évêque, 86600 Celle-Lévescault, France
[13]Present address: Aelin Therapeutics, Gaston Geenslaan 1, 3001 Leuven, Belgium
[14]These authors contributed equally
[15]Lead contact
*Correspondence: jhill@surfaceoncology.com (J.A.H.), savvas.savvides@ugent.be (S.N.S.)
https://doi.org/10.1016/j.celrep.2022.111490

SUMMARY

Interleukin-27 (IL-27) uniquely assembles p28 and EBI3 subunits to a heterodimeric cytokine that signals via IL-27Rα and gp130. To provide the structural framework for receptor activation by IL-27 and its emerging therapeutic targeting, we report here crystal structures of mouse IL-27 in complex with IL-27Rα and of human IL-27 in complex with SRF388, a monoclonal antibody undergoing clinical trials with oncology indications. One face of the helical p28 subunit interacts with EBI3, while the opposite face nestles into the interdomain elbow of IL-27Rα to juxtapose IL-27Rα to EBI3. This orients IL-27Rα for paired signaling with gp130, which only uses its immunoglobulin domain to bind to IL-27. Such a signaling complex is distinct from those mediated by IL-12 and IL-23. The SRF388 binding epitope on IL-27 overlaps with the IL-27Rα interaction site explaining its potent antagonistic properties. Collectively, our findings will facilitate the mechanistic interrogation, engineering, and therapeutic targeting of IL-27.

INTRODUCTION

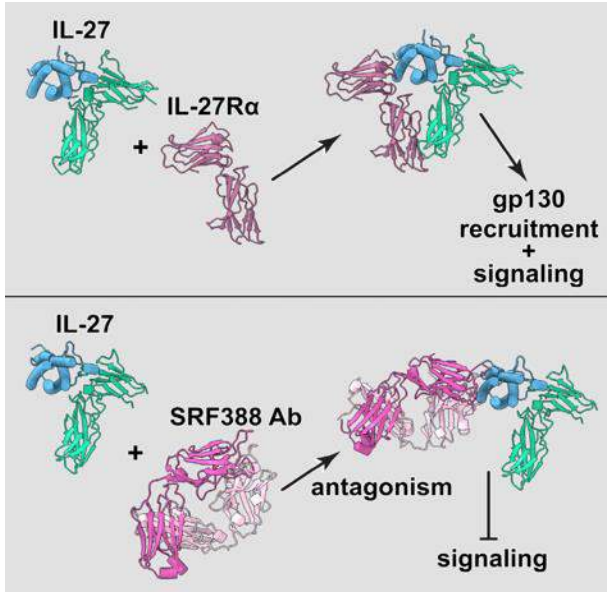
Interleukin-12 (IL-12) family cytokines (IL-12, IL-23, and IL-27, and the more recently reported IL-35 and IL-39) are distinguished by the pairing of their helical IL-6-like cytokine subunits (α-subunit) with soluble receptor chains (β-subunit), and the subsequent sharing of signaling receptors that regulate innate and adaptive immune responses in T cell populations (Hasegawa et al., 2016; Wojno et al., 2019). IL-27 is produced by activated antigen-presenting cells, such as dendritic cells and activated macrophages, and has emerged as perhaps the most unique member of the IL-12 family. IL-27 comprises a heterodimeric assembly of a p28 helical cytokine subunit with the compact soluble receptor Epstein-Barr virus-induced gene 3 (EBI3), respectively serving as the α- and β-cytokine subunits of a non-covalently linked heterodimeric cytokine. IL-27 signals through its specific cognate receptor IL-27Rα (also known as WSX-1 or TCCR) and the shared receptor gp130 (Pflanz et al., 2002, 2004) to drive Signal Transducer and Activator of Transcription (STAT) 1 and 3 signaling pathways (Pflanz et al., 2004; Wojno et al., 2019). The predicted structural homology of IL-27 with the archetypical IL-12 and IL-23 composite cytokines (that share a common p40 soluble receptor β-subunit), and the similarity of the p28 cytokine α-subunit with IL-6, imparted a pro-inflammatory skew to its ability to promote the production of interferon-γ (IFN-γ) by natural killer (NK) and T cells via Th1 responses. However, the currently understood functional landscape of IL-27 calls for a much broader influence on the inflammation spectrum due to its ability to modify CD4+ and CD8+ T cell effector functions, to promote T regulatory cell responses, and to orchestrate a suppressive transcriptional network (Andrews et al., 2016; Yoshida and Hunter, 2015). For instance, IL-27 is a potent inducer of the anti-inflammatory cytokine IL-10 (Awasthi et al., 2007; Fitzgerald et al., 2007; Stumhofer et al., 2007), which suppresses the development of Th17 cells



X-ray structure of SRF388 Fab in complex with IL-27

PDB code: 7ZXK

Skladanowska et al., 2022, Cell Reports, 41, 111490.



- IL-27Ra interacts both with the p28 and EBI3 subunits of IL- 27
- SRF388 and IL-27Ra occupy mutually exclusive binding sites on IL-27
- IL-27 mediates receptor assemblies distinct from IL-12 and IL-23

Activin ligand trap

iScience

CellPress
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Article

Structures of activin ligand traps using natural sets of type I and type II TGFβ receptors

Erich J. Goebel,[1] Chandramohan Kattamuri,[1] Gregory R. Gipson,[1] Lavanya Krishnan,[2] Moises Chavez,[2] Magdalena Czepnik,[1] Michelle C. Maguire,[2] Rosa Grenha,[2] Maria Håkansson,[3] Derek T. Logan,[3] Asya V. Grinberg,[4] Dianne Sako,[2] Roselyne Castonguay,[2] Ravindra Kumar,[2] and Thomas B. Thompson[1,5,*]

SUMMARY

The 30+ unique ligands of the TGFβ family signal by forming complexes using different combinations of type I and type II receptors. Therapeutically, the extracellular domain of a single receptor fused to an Fc molecule can effectively neutralize subsets of ligands. Increased ligand specificity can be accomplished by using the extracellular domains of both the type I and type II receptor to mimic the naturally occurring signaling complex. Here, we report the structure of one "type II-type I-Fc" fusion, ActRIIB-Alk4-Fc, in complex with two TGFβ family ligands, ActA, and GDF11, providing a snapshot of this therapeutic platform. The study reveals that extensive contacts are formed by both receptors, replicating the ternary signaling complex, despite the inherent low affinity of Alk4. Our study shows that low-affinity type I interactions support altered ligand specificity and can be visualized at the molecular level using this platform.

INTRODUCTION

The transforming growth factor β (TGFβ) family includes more than 30 structurally similar ligands that play essential roles in animals, regulating embryonic development, adult tissue homeostasis, immune system function, and metabolic pathways (Hinck, 2012; Hinck et al., 2016; Weiss and Attisano, 2013). The family can be divided into three main classes based on sequence homology and canonical Smad activation: the TGFβs, activins, and bone morphogenetic proteins (BMPs). In each case, signaling occurs when a hexameric signaling complex is assembled consisting of a dimeric ligand together with two type I and two type II serine/threonine kinase receptors (Attisano et al., 1993; Wrana et al., 1994). A striking feature of the family is that 30+ ligands share just seven type I and five type II receptors. Thus, an extensive network of promiscuous interactions exists between ligands and receptors, with each receptor typically binding multiple distinct ligands and for many ligands, multiple receptors (Goebel et al., 2019a). For example, activin class ligand, growth differentiation factor 11 (GDF11), can utilize activin-like kinase 4 (Alk4), Alk5, or Alk7 as type I receptors, while the closely related ligand activin A (ActA) is limited to Alk4.

Structural and biochemical studies have defined the major signaling paradigms for each of the three classes, distinguished by differential receptor binding interfaces and affinities (Allendorph et al., 2006; Goebel et al., 2019b; Groppe et al., 2008). These observations, together with evidence that the activins display remarkable structural flexibility in their type I receptor-binding interface, have led to a proposed model of conformational selection in which minor changes in the type I receptor-binding site play major roles in receptor selectivity (Goebel et al., 2019a, 2019b). While these previous studies began to clarify the mechanisms underlying the assembly of activin class ligands and their receptors, we lack a full understanding of how the activin class ligands achieve specificity for type I receptors, in large part due to a lack of molecular information of how Alk4 interacts with the ligands. In fact, no structures of Alk4 have yet to be described, which could detail how Alk4 engages multiple, distinct activin class ligands and enables mechanistic comparisons across the activin class as a whole.

TGFβ family ligands are attractive targets for therapeutic strategies due to their roles in many biological processes and diseases. For example, multiple members of the activin class negatively regulate skeletal muscle mass, prompting efforts to inhibit their combined signaling for therapeutic benefits in the context of muscle wasting conditions (Latres et al., 2017; Li et al., 2021; Puolakkainen et al., 2017). One strategy for

[1]Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati, 231 Albert Sabin Way ML 0524, Cincinnati, OH 45267, USA

[2]Acceleron Pharma, Inc., Cambridge, MA 02139, USA

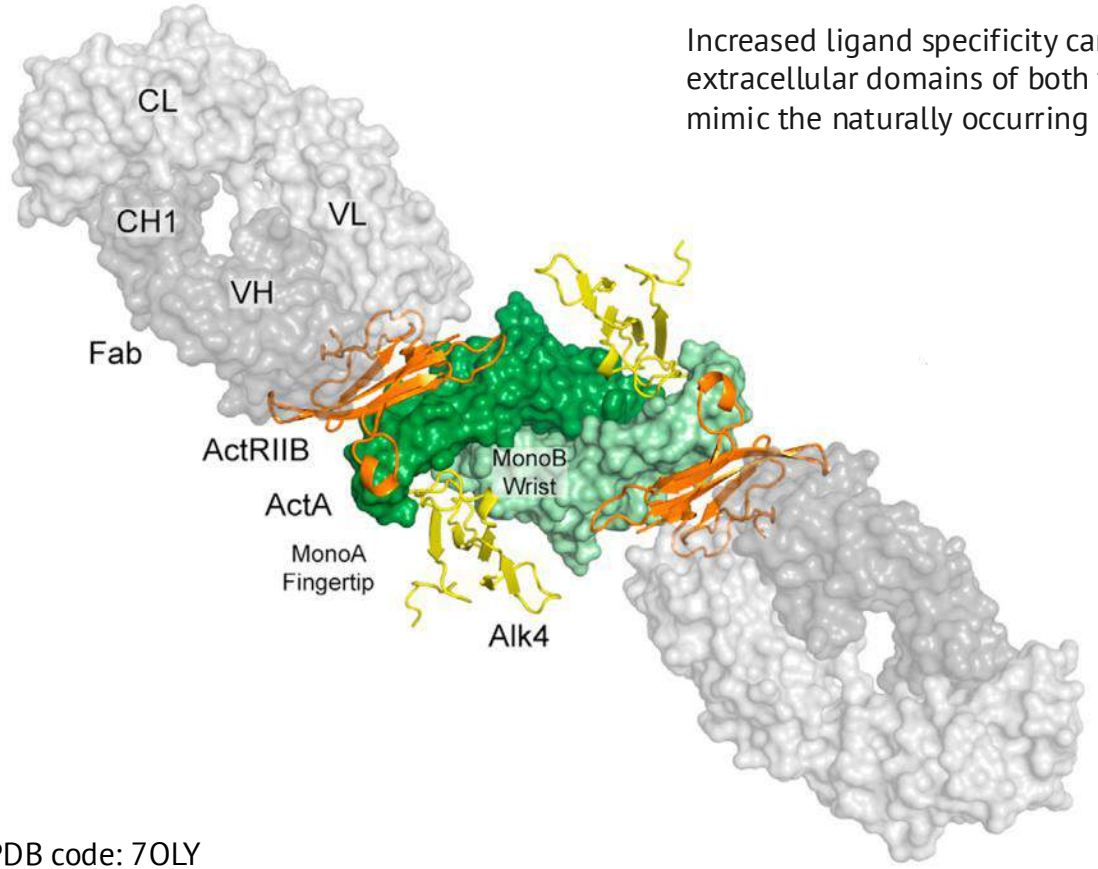
[3]SARomics Biostructures AB, Medicon Village, Scheelevägen 1, 223 63, Lund, Sweden

[4]Dragonfly Therapeutics, Waltham, MA 02451, USA

[5]Lead contact

*Correspondence: Tom.Thompson@uc.edu

https://doi.org/10.1016/j.isci.2021.103590



PDB code: 7OLY

Increased ligand specificity can be accomplished by using the extracellular domains of both the type I and type II receptor to mimic the naturally occurring signaling complex.

Structure of ActA/ActRIIB: Alk4/anti-ActRIIB Fab complex

Goebel et al., 2022, iScience, 25, 103590.

ATOR-1017 (evunzekibart), a 4-1BB agonist which activates exhausted T cells in combination with anti-PD-1

Cancer Immunology, Immunotherapy
https://doi.org/10.1007/s00262-023-03548-7

RESEARCH

ATOR-1017 (evunzekibart), an Fc-gamma receptor conditional 4-1BB agonist designed for optimal safety and efficacy, activates exhausted T cells in combination with anti-PD-1

Karin Enell Smith[1] · Sara Fritzell[1] · Anneli Nilsson[1] · Karin Barchan[1] · Anna Rosén[1] · Lena Schultz[1] · Laura Varas[1] · Anna Säll[1] · Nadia Rose[2] · Maria Håkansson[2] · Laura von Schantz[1] · Peter Ellmark[1,3]

Received: 6 August 2023 / Accepted: 11 September 2023

Abstract

Background 4-1BB (CD137) is a co-stimulatory receptor highly expressed on tumor reactive effector T cells and NK cells, which upon stimulation prolongs persistence of tumor reactive effector T and NK cells within the tumor and induces long-lived memory T cells. 4-1BB agonistic antibodies have been shown to induce strong anti-tumor effects that synergize with immune checkpoint inhibitors. The first generation of 4-1BB agonists was, however, hampered by dose-limiting toxicities resulting in suboptimal dose levels or poor agonistic activity.

Methods ATOR-1017 (evunzekibart), a second-generation Fc-gamma receptor conditional 4-1BB agonist in IgG4 format, was designed to overcome the limitations of the first generation of 4-1BB agonists, providing strong agonistic effect while minimizing systemic immune activation and risk of hepatoxicity. The epitope of ATOR-1017 was determined by X-ray crystallography, and the functional activity was assessed in vitro and in vivo as monotherapy or in combination with anti-PD1.

Results ATOR-1017 binds to a unique epitope on 4-1BB enabling ATOR-1017 to activate T cells, including cells with an exhausted phenotype, and NK cells, in a cross-linking dependent, FcγR-conditional, manner. This translated into a tumor-directed and potent anti-tumor therapeutic effect in vivo, which was further enhanced with anti-PD-1 treatment.

Conclusions These preclinical data demonstrate a strong safety profile of ATOR-1017, together with its potent therapeutic effect as monotherapy and in combination with anti-PD1, supporting further clinical development of ATOR-1017.

Keywords 4-1BB · CD137 · PD-1 · Immunotherapy · Antibody · T cell activation

Introduction

Immunotherapy using approved immune checkpoint inhibitors (ICI) has firmly established immuno-oncology as the fourth pillar of cancer therapy. Still, not all patients respond to ICI for multiple reasons, including absence or exhaustion of existing tumor-infiltrating lymphocytes, subverting their anti-tumoral properties. There is a need for improvement of current cancer immunotherapies by combining multiple immunomodulatory targeting regimens and developing novel therapies. Immunostimulatory antibodies targeting co-stimulatory receptors such as 4-1BB have been shown in preclinical models to induce synergistic effects with ICI, for example programmed cell death protein-1 (PD-1) [1, 2], and with radiotherapy [3] or chemotherapy [4].

4-1BB (CD137, TNFRSF9) is a co-stimulatory receptor transiently expressed on various immune cells, primarily on effector T cells upon antigen recognition through their T-cell receptor, but also on regulatory T cells (Treg) and natural killer (NK) cells [5, 6]. Importantly, 4-1BB is highly expressed on tumor infiltrating CD8 + T cells, cells with the capacity to specifically recognize and kill tumor cells, while 4-1BB expression on circulating T cells is low [7–9]. More specifically, 4-1BB is expressed on exhausted CD8 + T cells within the tumor microenvironment. These

Karin Enell Smith and Sara Fritzell have contributed equally to this work.

✉ Peter Ellmark
pek@alligatorbioscience.com

1 Alligator Bioscience AB, Lund, Sweden
2 Saromics Biostructures AB, Lund, Sweden
3 Department of Immunotechnology, Lund University, Lund, Sweden

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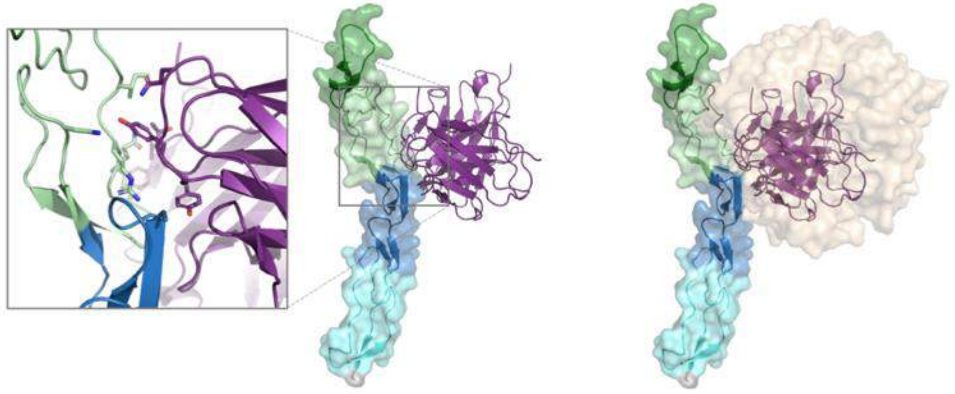
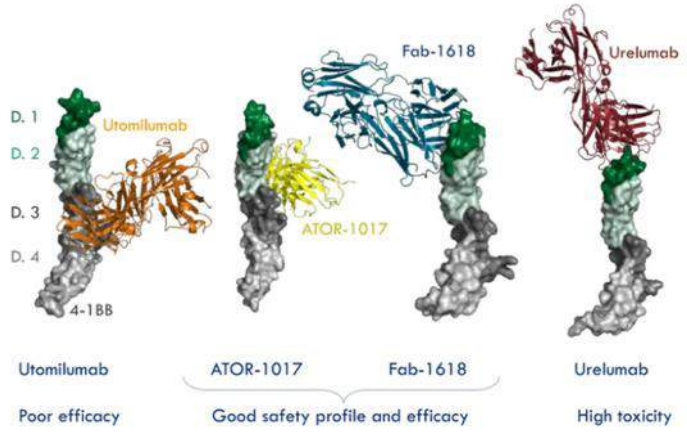
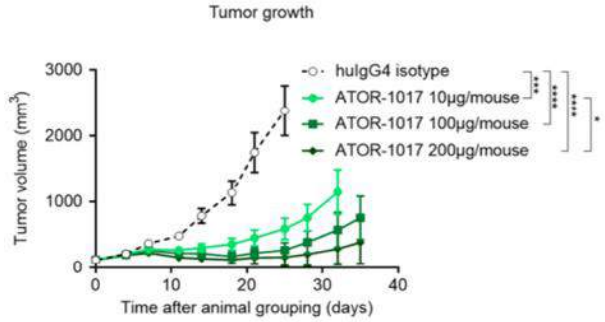


X-ray structure of ATOR-1017 in complex with 4-1BB (PDB code: 8OZ3)





- ATOR-1017 (evunzekibart), a second-generation Fc-gamma receptor conditional 4-1BB agonist, was designed to overcome the limitations of the first generation of 4-1BB agonists, providing strong agonistic effect while minimizing systemic immune activation and risk of hepatoxicity.
- ATOR-1017 binds to a unique epitope on 4-1BB enabling ATOR-1017 to activate T cells
- This translated into a tumor- directed and potent anti-tumor therapeutic effect in vivo, which was further enhanced with anti-PD-1 treatment

Enell Smith et al., 2023, Cancer Immunol, Immunother.

The bispecific 4-1BB x 5T4 agonist, ALG.APV-527, mediates strong T cell activation and potent anti-tumor activity

MOLECULAR CANCER THERAPEUTICS | LARGE MOLECULE THERAPEUTICS

The Bispecific Tumor Antigen-Conditional 4–1BB x 5T4 Agonist, ALG.APV-527, Mediates Strong T-Cell Activation and Potent Antitumor Activity in Preclinical Studies

Michelle H. Nelson[1], Sara Fritzell[2], Robert Miller[1], Doreen Werchau[2], Danielle Van Citters[1], Anneli Nilsson[2], Lynda Misher[1], Lill Ljung[2], Robert Bader[1], Adnan Deronic[2], Allison G. Chunyk[1], Lena Schultz[2], Laura A. Varas[2], Nadia Rose[3], Maria Håkansson[3], Jane Gross[1], Christina Furebring[2], Peter Pavlik[1], Anette Sundstedt[2], Niina Veitonmäki[2], Hilario J. Ramos[1], Anna Säll[2], Anna Dahlman[2], David Bienvenue[1], Laura von Schantz[2], Catherine J. McMahan[1], Maria Askmyr[2], Gabriela Hernandez-Hoyos[1], and Peter Ellmark[2,4]

ABSTRACT

4–1BB (CD137) is an activation-induced costimulatory receptor that regulates immune responses of activated CD8 T and natural killer cells, by enhancing proliferation, survival, cytolytic activity, and IFNγ production. The ability to induce potent antitumor activity by stimulating 4–1BB on tumor-specific cytotoxic T cells makes 4–1BB an attractive target for designing novel immuno-oncology therapeutics. To minimize systemic immune toxicities and enhance activity at the tumor site, we have developed a novel bispecific antibody that stimulates 4–1BB function when co-engaged with the tumor-associated antigen 5T4. ALG.APV-527 was built on the basis of the ADAPTIR bispecific platform with optimized binding domains to 4–1BB and 5T4 originating from the ALLIGATOR-GOLD human single-chain variable fragment library. The epitope of ALG.APV-527 was determined to be located at domain 1 and 2 on 4–1BB using X-ray crystallography. As shown in reporter and primary cell assays *in vitro*, ALG.APV-527 triggers dose-dependent 4–1BB activity mediated only by 5T4 cross-linking. *In vivo*, ALG.APV-527 demonstrates robust antitumor responses, by inhibiting growth of established tumors expressing human 5T4 followed by a long-lasting memory immune response. ALG.APV-527 has an antibody-like half-life in cynomolgus macaques and was well tolerated at 50.5 mg/kg. ALG.APV-527 is uniquely designed for 5T4-conditional 4–1BB–mediated antitumor activity with potential to minimize systemic immune activation and hepatotoxicity while providing efficacious tumor-specific responses in a range of 5T4-expressing tumor indications as shown by robust activity in preclinical *in vitro* and *in vivo* models. On the basis of the combined preclinical dataset, ALG.APV-527 has potential as a promising anticancer therapeutic for the treatment of 5T4-expressing tumors.

Introduction

Checkpoint inhibitor treatment has revolutionized cancer treatment resulting in sustainable clinical benefit. However, not all patients respond to this type of therapy, and there is need for additional improvements. A promising approach for cancer immunotherapy is to activate tumor-infiltrating T cells and natural killer (NK) cells through 4–1BB costimulation using antigen-conditional 4–1BB agonists (1).

4–1BB (TNF receptor superfamily 9, TNFRSF9, CD137) is a costimulatory receptor transiently expressed on various immune cells such as activated CD8 cytotoxic T cells, CD4 helper T cells, B cells, regulatory T cells, NK cells, natural killer T cells, and different myeloid cell populations (2). 4–1BB ligation provides the immune cell with a costimulatory signal that activates intracellular signaling cascades, for example, the NFκB pathway, inducing an increase in proliferation, cytokine production, and cytolytic activity (3, 4), and promoting T-cell survival and long-term protection from tumor recurrence (5). Although 4–1BB expression is low in naïve peripheral T cells, 4–1BB is highly upregulated upon T-cell receptor (TCR) activation on tumor-infiltrating T cells capable of killing tumor cells (6–9). In the immunosuppressive tumor microenvironment (TME), there is often a lack of costimulatory signals, such as the 4–1BB ligand (4–1BBL), limiting T-cell activation (10). Therefore, therapeutic antibodies targeting 4–1BB have been developed to mimic 4–1BBL stimulation to promote more effective antitumor responses. Although most 4–1BB agonistic mAbs require clustering via Fcγ receptors (FcγR) to activate 4–1BB, bispecific antibodies (bsAbs) use binding to antigens expressed in the TME to induce clustering and 4–1BB activation.

5T4 (trophoblast glycoprotein) is an oncofetal tumor-associated antigen (TAA) expressed in embryogenic trophoblasts. In adults, 5T4 has limited normal tissue expression restricted to some specialized

[1]Aptevo Therapeutics Inc., Seattle, Washington. [2]Alligator Bioscience AB, Lund, Sweden. [3]Saromics Biostructures AB, Lund, Sweden. [4]Department of Immunotechnology, Lund University, Lund, Sweden.

G. Hernandez-Hoyos and P. Ellmark contributed equally as co-authors of this work.

M.H. Nelson and S. Fritzell contributed equally as co-authors of this work.

Corresponding Author: Peter Ellmark, Alligator Bioscience, Medicon Village, 223 81 Lund, Sweden. Phone: 467-9721-2739; E-mail: pek@alligatorbioscience.com

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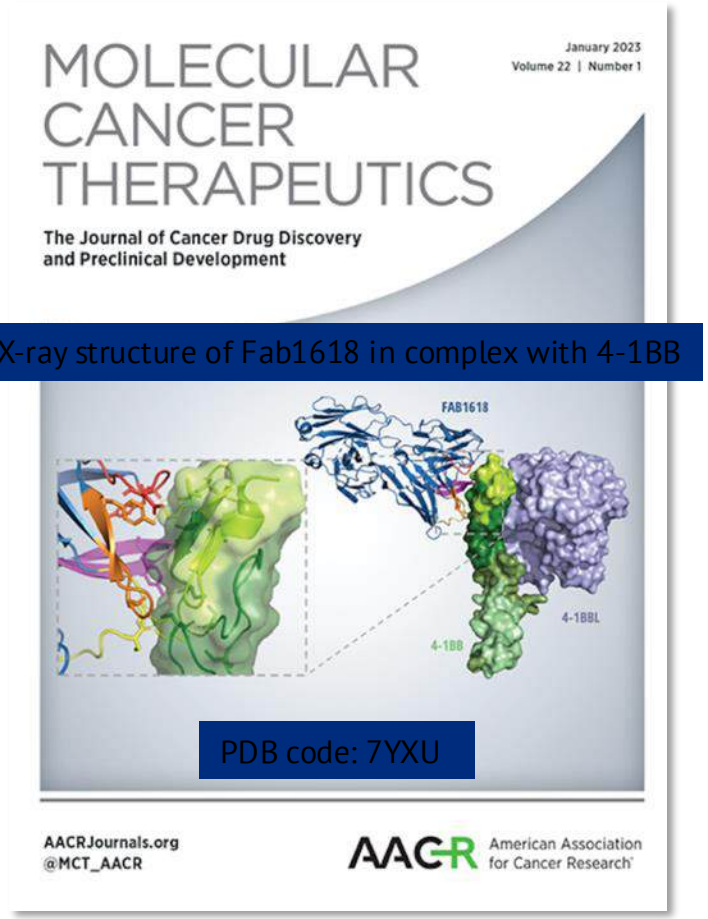

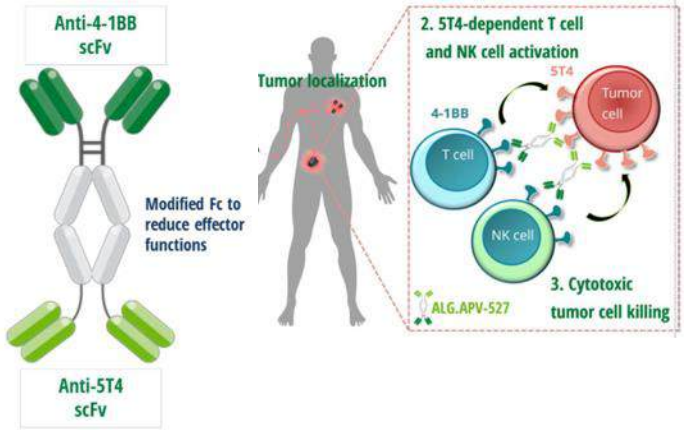

- ALG.APV-527 directs the stimulation of T cells and NK cells to 5T4+ tumors and is designed to minimize the toxicity observed with other 4-1BB therapeutics
- Binding sites of ALG.APV-527 and the 4-1BBL on 4-1BB are distinct

Nelson et al., 2023, Mol. Cancer Ther., 22-0395.

Targeting platelet GPVI with glenzocimab: a novel mechanism for inhibition

REGULAR ARTICLE

blood advances

Targeting platelet GPVI with glenzocimab: a novel mechanism for inhibition

Philippe Billiald,[1,2] Alexandre Slater,[3] Martin Welin,[4] Joanne C. Clark,[3] Stéphane Loyau,[1] Martine Pugnière,[5] Isabella G. Jacomini,[6] Nadia Rosa,[4] Kristell Lebozec,[7] Elie Toledano,[7] Déborah François,[7] Steve P. Watson,[3,8] and Martine Jandrot-Perrus[1]

[1]Laboratory for Vascular Translational Science, UMR_S1148 INSERM, Université Paris Cité, Hôpital Bichat, Paris, France; [2]School of Pharmacy, Université Paris-Saclay, Orsay, France; [3]Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom; [4]SARomics Biostructures, Medicon Village, Lund, Sweden; [5]Institut de Recherche en Cancérologie de Montpellier, INSERM, U1194, Université Montpellier, ICM Institut Régional du Cancer, Montpellier, France; [6]Departamento de Patologia Básica, Laboratório de Imunoquímica, Universidade Federal do Paraná, Curitiba, Brazil; [7]Acticor Biotech, Hôpital Bichat, Paris, France; and [8]Centre of Membrane Proteins and Receptors, Universities of Birmingham and Nottingham, Midlands, United Kingdom

Key Points

- Crystallization studies map the binding of glenzocimab to the site of dimerization in the D2 domain of GPVI.
- Glenzocimab inhibits GPVI interactions with CRP, collagen, and fibrin by loss of dimerization, conformational changes, and steric hindrance.

Platelet glycoprotein VI (GPVI) is attracting interest as a potential target for the development of new antiplatelet molecules with a low bleeding risk. GPVI binding to vascular collagen initiates thrombus formation and GPVI interactions with fibrin promote the growth and stability of the thrombus. In this study, we show that glenzocimab, a clinical stage humanized antibody fragment (Fab) with a high affinity for GPVI, blocks the binding of both ligands through a combination of steric hindrance and structural change. A cocrystal of glenzocimab with an extracellular domain of monomeric GPVI was obtained and its structure determined to a resolution of 1.9 Å. The data revealed that (1) glenzocimab binds to the D2 domain of GPVI, GPVI dimerization was not observed in the crystal structure because glenzocimab prevented D2 homotypic interactions and the formation of dimers that have a high affinity for collagen and fibrin; and (2) the light variable domain of the GPVI-bound Fab causes steric hindrance that is predicted to prevent the collagen-related peptide (CRP)/collagen fibers from extending out of their binding site and preclude GPVI clustering and downstream signaling. Glenzocimab did not bind to a truncated GPVI missing loop residues 129 to 136, thus validating the epitope identified in the crystal structure. Overall, these findings demonstrate that the binding of glenzocimab to the D2 domain of GPVI induces steric hindrance and structural modifications that drive the inhibition of GPVI interactions with its major ligands.

Introduction

The finely tuned formation of a platelet plug at sites of vascular injury ensures hemostasis by preventing excessive blood loss. By contrast, uncontrolled platelet activation causes thrombotic events and acute ischemic events such as myocardial infarction or stroke. Moreover, platelets and immune cells act jointly in injured tissues, leading to thromboinflammation that contributes to cell death and organ dysfunction.[1] Antiplatelet drugs are largely used for the treatment and prevention of arterial thrombosis, including

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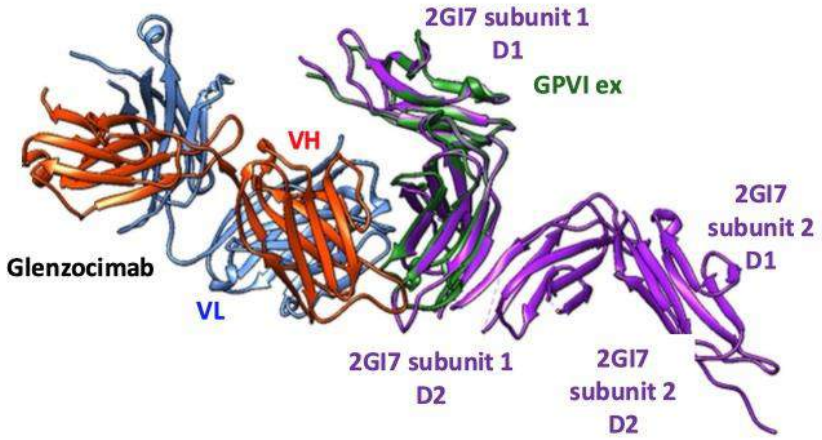
Atomic coordinates and structure factors (PDB ID codes 7R58) have been deposited in the Protein Data Bank (www.wwpdb.org).

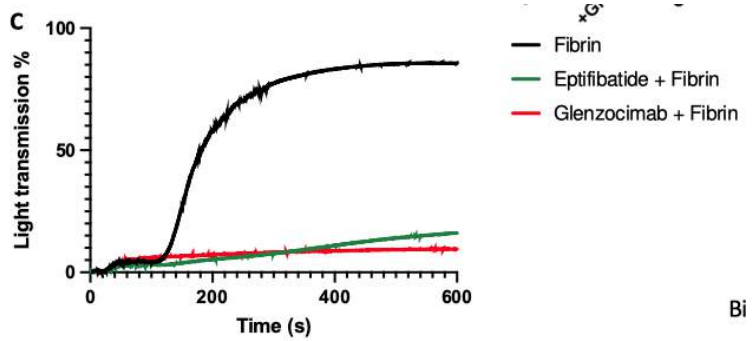
Data are available on request from the corresponding authors, Martine Jandrot-Perrus (martine.jandrot-perrus@inserm.fr) and Philippe Billiald (philippe.billiald@universite-paris-saclay.fr).

The full-text version of this article contains a data supplement.



X-ray structure of glenzocimab in complex with GPVI

PDB code: 7R58



Glenzocimab inhibits fibrin-induced platelet aggregation

- GPVI binding to vascular collagen initiates thrombus formation and GPVI interactions with fibrin promote the growth and stability of the thrombus.
- Crystal structure information enables the **elucidation of a novel mechanism** for the powerful anti thrombotic effect of glenzocimab, in which both ligands are blocked through a combination of steric hindrance and structural change.

Billiald et al., 2022, Blood Adv (2023) 7 (7): 1258–1268.

DutaFabs - engineered Fab's that bind two antigens simultaneously

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-20949-3 OPEN

DutaFabs are engineered therapeutic Fab fragments that can bind two targets simultaneously

Roland Beckmann[1✉], Kristian Jensen[1,4], Sebastian Fenn[1], Janina Speck[1], Katrin Krause[1], Anastasia Meier[1], Melanie Röth[1], Sascha Fauser[2], Raymond Kimbung[3], Derek T. Logan[3], Martin Steegmaier[1] & Hubert Kettenberger[1]

We report the development of a platform of dual targeting Fab (DutaFab) molecules, which comprise two spatially separated and independent binding sites within the human antibody CDR loops: the so-called H-side paratope encompassing HCDR1, HCDR3 and LCDR2, and the L-side paratope encompassing LCDR1, LCDR3 and HCDR2. Both paratopes can be independently selected and combined into the desired bispecific DutaFabs in a modular manner. X-ray crystal structures illustrate that DutaFabs are able to bind two target molecules simultaneously at the same Fv region comprising a VH-VL heterodimer. In the present study, this platform is applied to generate DutaFabs specific for VEGFA and PDGF-BB, which show high affinities, physico-chemical stability and solubility, as well as superior efficacy over anti-VEGF monotherapy in vivo. These molecules exemplify the usefulness of DutaFabs as a distinct class of antibody therapeutics, which is currently being evaluated in patients.

[1] Roche Pharma Research and Early Development, Roche Innovation Center Munich, Roche Diagnostics GmbH, Penzberg, Germany. [2] Roche Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland. [3] SARomics Biostructures AB, Medicon Village, Lund, Sweden. [4] Present address: Bornspire, Copenhagen, Denmark. ✉email: pred.dutafabs@roche.com

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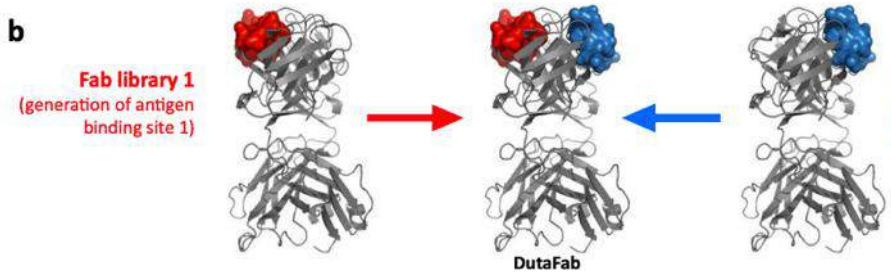
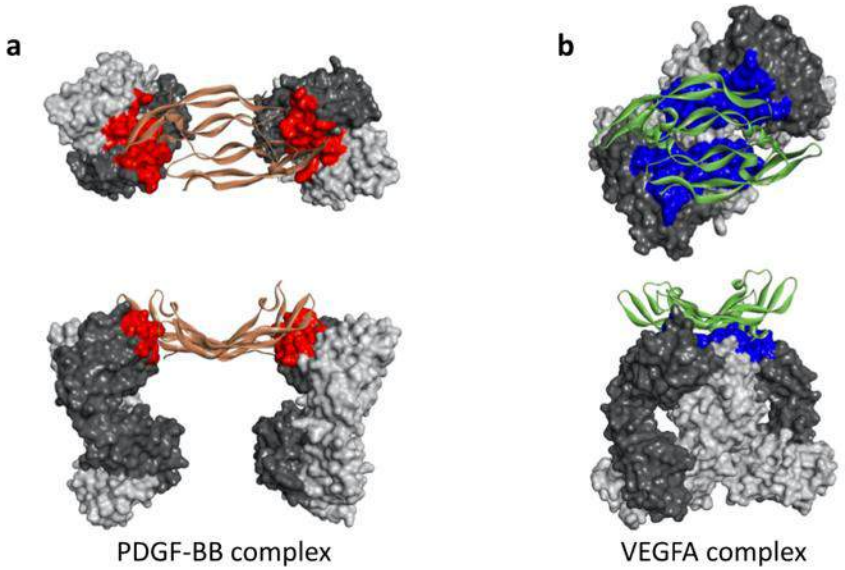


The DutaFab concept of separating paratopes on a single Fab



X-ray structure of the DutaFab in complex with PDGF-BB dimer and VEGFA dimer

PDB code: 6T9E 6T9D

Beckmann et al., 2021, Nat Comm, 12:708.