The 4-1BB (CD137) Costimulatory Pathway as a Therapeutic Target

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Factors determining T cell antitumor responses

- TCR/MHC interaction triggers T cell activation
- Co-inhibitory receptor/ligand interactions limit magnitude and duration of immune response
- Co-stimulatory receptor/ligand interactions enhance magnitude and duration of immune response
- Therapeutic targeting of co-inhibition is straightforward: simple antagonism: anti-CTLA-4 and anti-PD-1 mAbs show impressive clinical results
- Therapeutic exploitation of (co-)stimulation is complex
  - High efficacy at low toxicity not yet achieved for costimulatory immune receptor agonists

Differences and Commonalities in the TNFR Costimulatory Family

- TNF receptor family and TNFR ligands highly relevant for costimulation on lymphocytes, especially T cells
- Other family members also relevant in immunology
- Differences between TNFR family members
  - Expression of receptor (e.g. CD4/CD8 T cells, T reg, NK, APC)
  - Expression of ligand (e.g. T cells, various APC)
  - Downstream signaling
- Activation of costimulatory TNFR on effector lymphocytes enhances their
  - Proliferation and survival
  - Proinflammatory cytokine production
  - Cytotoxic function
  - Memory formation

→ Universal importance of TNFR family in immunology makes it an attractive target class

4-1BB (CD137) is a preclinically validated target with clinical pathway validation

- **4-1BB profile**
  - Expressed on activated CD4+ and CD8+ T cells, activated B cells, and NK cells
  - The only known ligand 4-1BBL is expressed on various types of antigen-presenting cells
  - 4-1BB activation leads to strong costimulation of TCR-activated T cells

- **4-1BB targeting leads to tumor rejection in mouse models**
  - Forced expression of 4-1BBL on tumor
  - Forced expression of an anti-4-1BB scFv on tumor
  - Systemic anti-4-1BB antibody (retardation of tumor growth)

- **Human clinical data supports relevance of 4-1BB**
  - 4-1BB is a validated marker for tumor-reactive T cells in man
  - Anti-4-1BB mAbs improve expansion of CD8+ melanoma TIL in adoptive T cell therapy
  - 4-1BB downstream signaling is key to success in clinical CAR-T
  - 4-1BB expression is localized in the tumor microenvironment

- **Tumor-targeted T cell engagement via 4-1BB has been demonstrated using aptamer technology**

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General Mechanism of Co-stimulation by TNFR such as 4-1BB

- TNFR family receptors require clustering for activation
- Soluble ligands are usually not sufficient to activate TNFR family receptors
- Without further clustering, bivalent mAbs are also not sufficient to activate

Mechanism of TNFR activation supports tumor-target based activation approach
Concept: Tumor-localized Co-stimulation with Bispecific 4-1BB Engager

- **No activation**

- **Clustering**
  - 4-1BB is activated via higher order clustering
  - Tumor receptor mediated clustering of bispecifics drives 4-1BB-mediated T cell activation
  - Maintained tumor antigen specificity by T cell receptor leading to potential safety advantages

- **Activation**

- **No T cell costimulation in periphery**
  - No T cell costimulation in periphery

- **T cell costimulation in tumor**
  - T cell costimulation in tumor
The Anticalin Platform as the Basis for Fit-For-Purpose Costimulatory Bispecifics

- **Anticalins** are a novel class of protein therapeutics, proprietary to Pieris, with several degrees of validation.
- Human data demonstrating desired drug-like properties:
  - 26 solid tumor patients with VEGF-A antagonist
  - 36 healthy volunteers with hepcidin antagonist
- Proven track record for successful collaborations with Pharma:
  - Roche
  - Sanofi
  - Daiichi-Sankyo
  - Allergan
  - Zydus
  - Stelis Biopharma
Anticalins are a Novel Class of Therapeutic Binding Proteins

- Anticalins® are derived from lipocalins – human extracellular binding proteins
- Small (18 kDa vs 150 kDa mAbs), high selectivity and potency

**Anticalins Have Been Generated Against a Broad Range of Targets**

- Anticalin in complex with a small molecule (Y-DTPA)
- Anticalin bound to the hepcidin peptide
- Anticalin bound to the CTLA4 protein

Human lipocalin
Red = key areas of target engagement
Anticalin-Based Drug Candidates Can Be Tailored to Multiple Formats

Potent Multi-target Engagement  |  Novel MoA  |  Excellent Drug-like Properties
PRS-343: HER2 Targeted 4-1BB Activation

4-1BB (CD137) – Key TNFR Family Costimulatory Target
- Marker for tumor-specific T cells in TME
- Pathway clinically validated in CAR-T and ACT
- Strong preclinical validation in mouse tumor models (forced ligand expression, mAb therapy)
- Critical for sustained T cell survival and activity

HER2 – Strongly Validated Tumor Target
- Restricted expression on normal tissue
- Differential expression supports PRS-343 drug trafficking and cross-linking at the tumor bed
- Multiple HER2+ tumors with high-unmet need
  - Breast, Gastric, Esophageal, Bladder, Ovarian, Endometrial, Lung (AdenoCa), Biliary, Salivary Duct
PRS-343 was chosen from multiple variants generated by versatile platform

**4-1BB-targeting Anticalin**
- Affinity 2nM (SPR)
- Non-competitive mode of binding
- Activates 4-1BB only when coated/clustered

**HER2-targeting: engineered trastuzumab**
- Engineered IgG4 backbone
- FcgR-interaction nearly eliminated

**Selection criteria:**
- Target binding (in vitro and FACS)
- FcgR and FcRn interaction
- Plasma and storage stability
- Mouse and cynomolgus monkey PK
- Ex vivo T cell activation
- Activity in tumor models in vivo

- PRS-343 was selected from multiple variants
- All variants showed retained target binding and excellent drug-like behavior
- Key selection criterion was activity in ex vivo T cell activation and mouse tumor model
PRS-343 Leads to T cell Costimulation - Bispecific Geometry is Crucial

4-1BB/HER2 bispecific variants induce T cell activation with different potency, demonstrating the importance of bispecific geometry.
T Cell Activation is HER2 Target-Dependent

Addition of excess HER2-targeting trastuzumab prevents binding of 4-1BB/HER2 bispecifics to HER2-positive cells and results in a loss of activity, confirming mode of action.
PRS-343 Activates 4-1BB in Tumor Target-dependent Manner, Distinct From Benchmark Antibodies

- PRS-343 selectively activates T cells in the presence of HER2-high cells
- This mode of action is markedly different to 4-1BB-targeting benchmark 1
- 4-1BB targeting benchmark 2 shows no activity, highlighting the need for higher order clustering
- Mode of action supports low expected toxicity against healthy cells
PRS-343 Tested in SK-OV-3 Humanized Mouse Model – Experimental Protocol

Groups
- PBMC control
- Vehicle control
- Isotype control
- PRS-343 (200µg)
- PRS-343 (100µg)
- PRS-343 (20µg)
- PRS-343 (4µg)
- Tras-IgG4
- Anti-4-1BB benchmark (100µg)

Tumor engraft (s.c.)
PBMC engraft (2 donors) D0 (i.v.)
TIL analysis (IHC of tumors) D20

Readouts
- Tumor size
- Body weight
- Survival
- Lymphocyte phenotyping at study end
- For select animals: HE & CD45 IHC

Notes
- Immune-compromised NOG mice
- Tumor size at day of PBMC engraftment: approx 120 mm³
PRS-343 shows bifunctional activity – dose-dependent tumor growth inhibition & CD8(+)TIL expansion in SK-OV-3 model

- PRS-343 shows dose-dependent tumor growth inhibition, which is dominated by anti-HER2 activity
- PRS-343 leads to strong and dose-dependent lymphocyte infiltration in tumors; monospecific anti-HER2 mAb (IgG4 backbone) lacks this activity
- Monospecific anti-4-1BB benchmark mAb shows insignificant response compared to isotype control and no significant tumor infiltration of lymphocytes

**Tumor growth (Median)**

- no PBMC
- PBMC only
- Anti-CD137 100µg
- Isotype ctrl 100µg
- PRS-343 4µg
- PRS-343 20µg
- Tras-IgG4 80µg

**TIL frequency (hCD45)**

- Incomplete group due to mortality
Anti-4-1BB mAb (but not PRS-343) Expands Peripheral Lymphocytes and accelerates GvHD

- Anti-4-1BB benchmark mAb shows accelerated GvHD with significant mortality at day 20 in line with literature data
- Toxicity corresponds with expansion of CD8-positive T cells in PBMC for this group

1 GvHD = graft vs host disease
2 Sanmamed et al., Cancer Res. 2015 Sep 1;75(17):3466-78.
PRS-343: Excellent drug-like properties

- Full plasma stability
  - Fully active after 1 week in human and mouse plasma at 37°C (0.5mg/mL)

- Excellent storage stability
  - Fully stable and active after 4 weeks at 40°C in PBS (20mg/mL)

- Antibody-like half-life in mice and cynomolgus monkey
  - Half-life similar to trastuzumab in mice (10mg/kg) and cyno (3mg/kg)

- Low Immunogenicity Risk
  - According to in vitro T cell immunogenicity experiment (Epibase, Lonza)

- Good manufacturability
  - High titer expression up to 4 g/L in established CMC process
Summary

- Immunostimulation requires bispecifics for **efficacy**
  - 4-1BB/HER2 bispecific PRS-343 shows strong tumor-localized T cell activation in vivo
  - Anti-4-1BB benchmark activates T cells in periphery but not in the tumor

- Immunostimulation requires bispecifics for **safety**
  - Systemic activation of 4-1BB by anti-4-1BB mAb leads to increased toxicity in vivo

- Pieris bispecifics platform generates fit-for-purpose multispecifics
  - Flexible geometry, valency and affinity
  - Excellent drug-like properties: stability, PK, immunogenicity, manufacturability
  - Tailor-made drugs for cancer immunotherapeutics and other indications

- PRS-343 is advancing to the clinic based on excellent preclinical POC
  - IND-enabling studies progressing
  - First-in-patient trial in HER2-positive solid tumors unresponsive to SOC in H1 2017

- Multispecific costimulatory approach broadly applicable to multiple tumor targets and costimulatory receptors – 2nd disclosed program PRS-342 (4-1BB/GPC-3 bispecific)
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