Abstract #4867

A FAS-based switch receptor tailored to PRAME positive cancer indications, engineered to boost T cell engraftment and anti-tumor activity

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Background

Adoptive cell therapies have shown promising efficacy in melanoma and synovial sarcoma, but success in other solid tumor types remains limited. A significant challenge is the hostile tumor microenvironment (TME) that restricts T cell infiltration, function and persistence. Here we developed a switch receptor (SwR) designed to convert inhibitory signals operating in PRAME positive solid tumors into pro-activation signals to support T cell engraftment and fitness and prevent T cell death within the harsh TME.

Methods

- Expression of the mRNAs encoding FAS, PD1 and TIGIT ligands in healthy tissues and in tumors was analyzed in bulk and at single-cell level using public and proprietary transcriptomics datasets.
- A FAS-SwR was developed through a stepwise process involving sequential selection and optimization steps. Evaluation of FAS-SwRs was performed using T cells retrovirally transduced to express different SwRs in combination with an HLA-A*0201 restricted MAGE-A1 or PRAME-specific TCR with or without a CD8 co-receptor. T cells were tested for proliferation, viability, cytotoxicity and cytokine secretion using FAS-L expressing lung cancer cell lines. Cytokine secretion was measured using a multiplex cytokine bead array.

FAS ligand is the only inhibitory ligand expressed in all indications while having favorable expression in healthy tissue

A

Receptor	Ligand	MEL	UC	OEC	LUSC	TNBC	LUAD	HNSCC	ESCA	iBLCA	CECA
FAS	FAS-L										
PD1	CD274										
TIGIT	PVR										
	NECTIN2										
	NECTIN4										

Ligand mRNA (median log_2(TPM+1)): 0 – 7.5

CECA: cervical cancer; ESCA: esophageal cancer; HNSCC: head and neck squamous cell carcinoma; iBLCA: invasive bladder carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; MEL: melanoma; OEC: ovarian epithelial carcinoma; TNBC: triple-negative breast carcinoma; TPM: transcript per million; UC: uterine cancer.

B

		Testis*				Ovary*		Endometrium		Kidney	
Receptor	Ligand	Spermatogonia	Spermatocytes	Early spermatids	Late spermatids	Granulosa cells	Oocyte	Ciliated cells	Glandular and luminal cells	PTECs	Adrenal glands
FAS	FAS-L										
PD1	CD274										
TIGIT	PVR										
	Nectin2										
	Nectin4										

Ligand mRNA (log_2(nTPM+1)): 0 – 7

* Immune privileged sites

TPM: transcript per million. PTECs: proximal tubular epithelial cells.

Fig. 1: (A) FAS, PD1 and TIGIT ligands are expressed in all PRAME-expressing indications investigated, with TIGIT ligands showing the highest levels. Ligand expression was analyzed in 4622 de-identified patient records of patients across 10 cancer indications whose samples underwent comprehensive genomic and transcriptomic profiling with the Tempus xT and xR next-generation sequencing assays (Tempus AI, Inc.) (n=84-1421/indication).
(B) FAS ligand shows favorable expression in healthy tissues, while PD1 and TIGIT ligands are expressed at moderate or high levels in multiple healthy tissues, including cell types where low PRAME expression has been reported. HPA v24.0 single-cell RNAseq data for all cell types except adrenal glands, for which single-cell RNAseq data were not available and consensus bulk RNAseq data are shown.

FAS ligand is expressed by T and NK cells in healthy tissues and in tumors

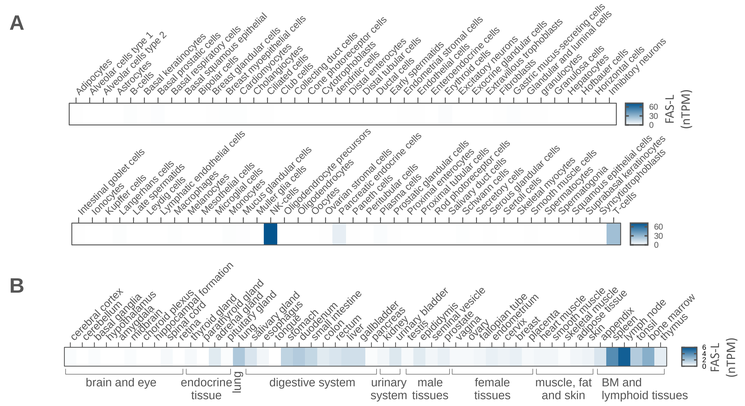


Fig. 2: (A,B) In healthy tissues, FAS ligand (FAS-L) mRNA is exclusively expressed by T and NK cells, and the highest levels of FAS-L mRNA are found in secondary lymphoid organs. (A) Analysis of the HPA single cell RNAseq dataset. Signal in cell types other than T and NK cells is considered background (<5 reads). **(B)** Analysis of HPA consensus RNAseq dataset.
(C, D) T cells constitute the key source of FAS-L in tumors. (C) Single-cell RNAseq data obtained from publications on 3-9 tumors from five PRAME-positive indications. **(D)** Transcriptomics analysis of 4622 tumor samples from the Tempus database showed positive correlation of FAS ligand expression and T cell content. The correlation was observed for each of the 10 indications (cf Fig.1) and treatment groups (cf Fig. 3A).

FAS ligand expression is increased after treatment with immune checkpoint inhibitors, but minimally influenced by line of treatment and tumor location

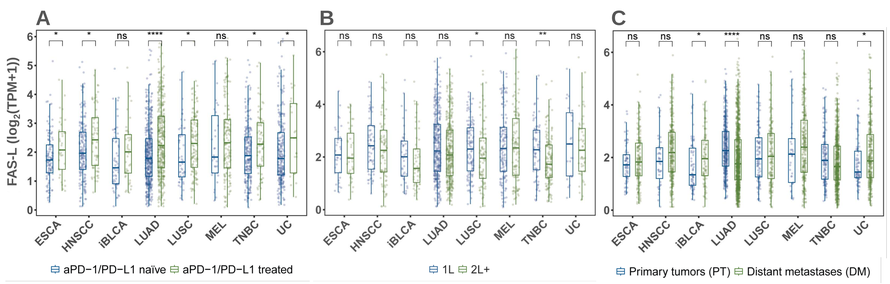


Fig. 3: Analysis of FAS-L expression in 4622 tumors from PRAME-expressing indications (Tempus database)
(A) FAS-L levels are higher in patients having received anti-PD-1 and/or anti-PD-L1 treatment. Data shown from tumors at first line of therapy (1L). Similar results were obtained for tumors collected in later lines.
(B) FAS-L levels are higher or similar in first compared to later lines of treatment. Data shown from tumors after anti-PD-1 and/or anti-PD-L1 treatment. Similar results were obtained in tumors naïve to anti-PD-1/anti-PD-L1.
(C) FAS-L levels are similar between primary tissues and distant metastases, except in LUAD.
Only groups with at least 20 records are shown. Boxes correspond to 25th, 50th and 75th percentiles; whiskers go from min to max. p-values above the box plots were obtained using Wilcoxon's rank sum statistical tests. ns: not significant (p-value>0.05); *p-value<0.05; **p-value <0.01; ***p-value<0.001

FAS-TNFR is designed to prevent exhaustion and apoptosis in TME, promoting engraftment and durable anti-tumor responses

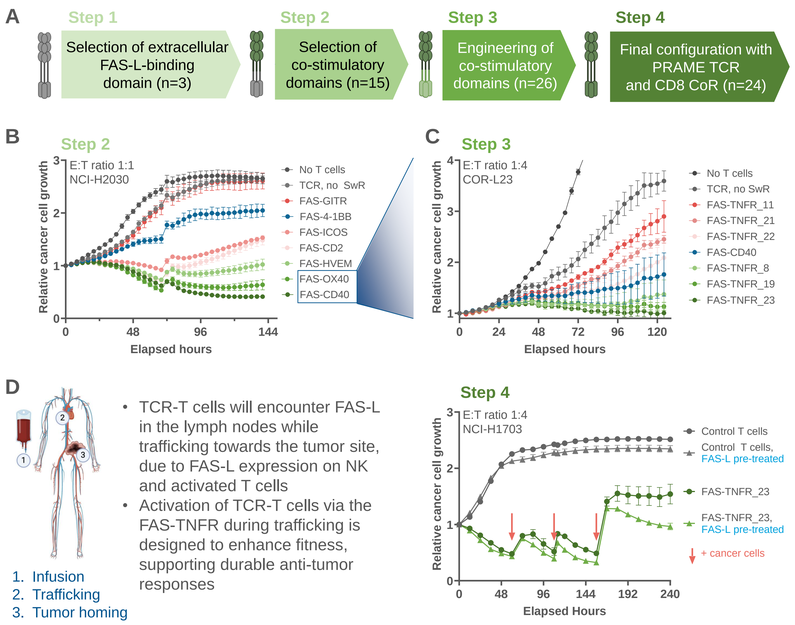
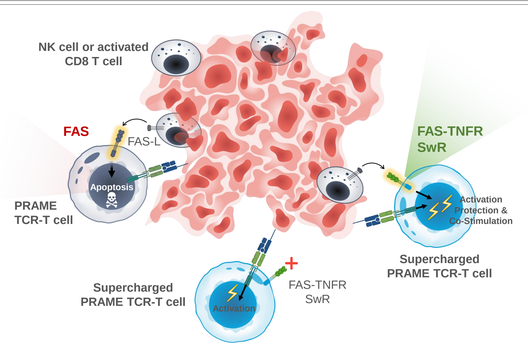


Fig. 4: FAS-TNFR SwR is designed to prevent exhaustion and apoptosis in TME, promoting engraftment and durable anti-tumor responses. (A) Selection funnel for FAS-SwR. **(B)** FAS-CD40 and FAS-OX40 mediated best resistance to apoptosis and superior cytotoxicity in a co-culture with FAS-L expressing cancer cells. Seven out 15 candidates tested are shown. **(C)** Further engineering combining CD40 and OX40 motifs resulted in selection of FAS-TNFR mediating durable anti-tumor responses at low E:T ratio. Seven out of 26 designs are shown. (**D**) FAS-L pre-stimulation (+FAS-L) that mimics exposure during TCR-T cell trafficking (left panel) enhances serial killing activity of TCR-T cells bearing FAS-TNFR (right panel).



Conclusions

Extensive characterization of the TME of PRAME-positive indications guided the development of a best-in-class FAS-TNFR switch receptor ("checkpoint converter"). Based on these data, FAS-TNFR is expected to deepen clinical responses at lower doses and result in durable anti-tumor responses, because it will:

- **Support engraftment and fitness of PRAME TCR-T cells before tumor homing**
- **Prevent T-cell death and boost durable anti-tumor activity within harsh TME**
- **Decrease soluble FAS-L locally protecting other immune cells from apoptosis** #3198