Affinity tuned XmAb® 2+1 GPC3 x CD3 bispecific antibodies demonstrate selective activity in liver cancer models

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Abstract #1831

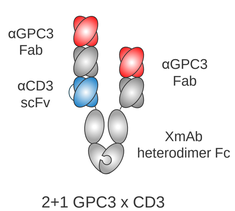
Introduction

- Glypican 3 (GPC3) is differentially expressed in several cancers, but especially in hepatocellular carcinoma (HCC) and lung squamous cell carcinoma (LSCC) as measured by bulk RNAseq and IHC.
- A lipid anchored and heparan sulfate-containing cell surface protein, GPC3 is an intriguing target as it serves as a reservoir for Wnt, and under specific conditions can trigger Wnt signaling, increase β-catenin expression, and promote tumor proliferation.
- GPC3's expression is important during embryonic development, but it is heavily suppressed in adult tissues. Despite its favorable expression profile, toxicity and/or CRS have been reported from 1st gen efforts with CAR-T and T cell engaging bispecific antibodies.
- Bispecific T cell engagers are powerful immunomodulatory agents that benefit from careful tuning to improve the therapeutic window. To create a selective T cell engaging antibody against GPC3, we extended our XmAb heterodimeric Fc platform to create the 2+1 Fab$_2$-scFv-Fc format, which is bivalent for GPC3 and monovalent for CD3.

A XmAb 2+1 Fab$_2$-scFv-Fc format enables selective tumor targeting

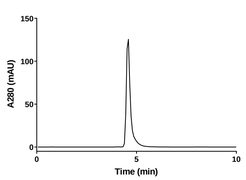
XmAb heterodimeric Fc platform allows for well-behaved, high-yielding, and easily manufactured 2+1 bispecific antibodies

- Modified Fc domain eliminates FcγR reactivity
- Preserved FcRn affinity can be enhanced with Xtend Fc technology to promote even longer half-life
- Fc substitutions promote heterodimer formation and facilitate purification by standard methods such as Protein A + ion-exchange chromatography
- An αGPC3 Fv was humanized, affinity-tuned, and inserted into our CD3 bispecific platform.

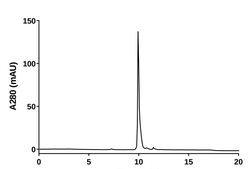


2+1 GPC3 x CD3

Favorable solution properties (SEC)



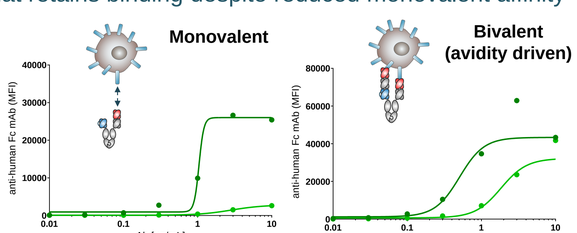
High purity bispecific (IEX)



B Bivalent 2+1 format retains binding despite reduced monovalent affinity

Decreasing GPC3 affinity

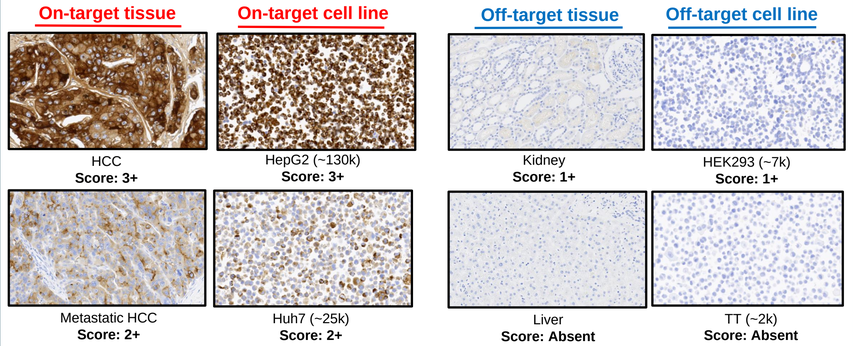
- Cell binding performed on HepG2 (~130K)



C Cell line proxies for "on-target" and "off-target" tissue identified by IHC

- GPC3 prevalence was measured by IHC scoring of tumor and normal tissue FFPE cores
- Antigens per cell on various endogenous GPC3+ cell lines ranged from ~130K to ~7K, and were correlated against tumor and normal tissues by IHC

		3+	2+	1+	Absent
	(n)	% (n)	% (n)	% (n)	% (n)
Hepatocellular carcinoma (HCC)	30	43% (13)	20% (6)	17% (5)	20% (6)
Metastatic HCC	5	20% (1)	40% (2)	0% (0)	40% (2)
Normal Tissue	99	0% (0)	0% (0)	15% (15)	85% (84)

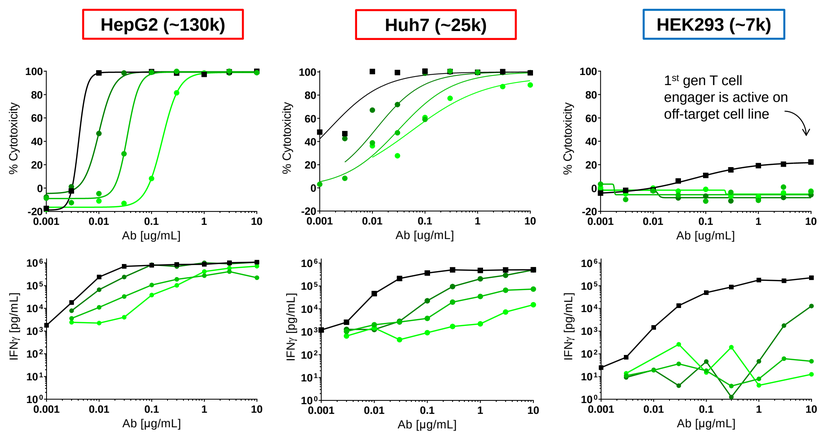


D Tuned 2+1 bispecifics selectively kill on-target cell lines and avoid off-target cell line in vitro

- Cell lines were mixed with PBMCs at E:T of 1:1, then treated with antibodies for 72 hrs

1st gen T cell engager (high affinity for both GPC3 and CD3)

Tuned 2+1 bispecifics with decreasing potency



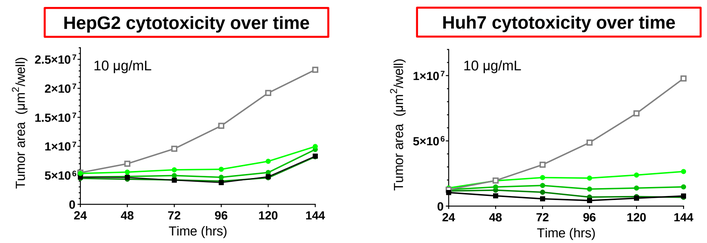
E Tuned CD3 bispecifics are efficacious in longitudinal in vitro liver models

1 Tuned CD3 bispecifics match the efficacy of a 1st gen T cell engager in 2D models

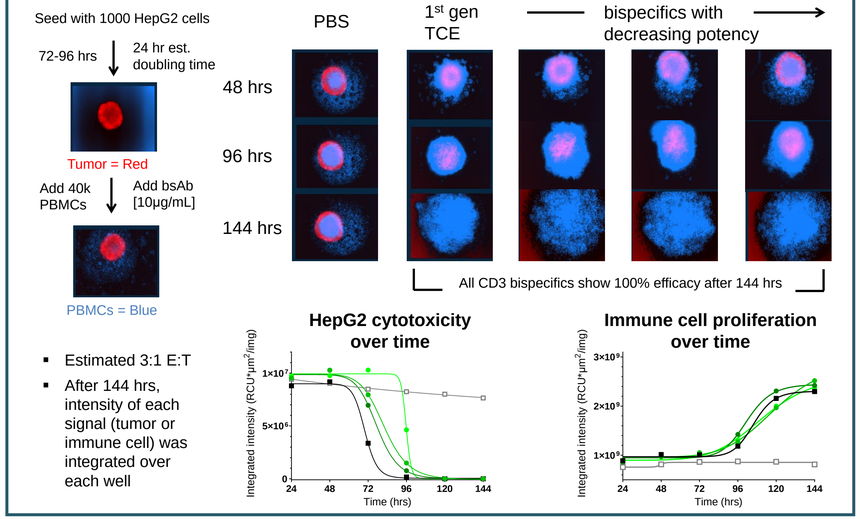
PBS

1st gen T cell engager (high affinity for both GPC3 and CD3)

Tuned CD3 bispecifics with decreasing potency



2 Tuned CD3 bispecifics match efficacy and immune cell proliferation of a 1st gen T cell engager in 3D spheroid models



- Estimated 3:1 E:T
- After 144 hrs, intensity of each signal (tumor or immune cell) was integrated over each well

HepG2 cytotoxicity over time

Immune cell proliferation over time

Summary

Tuned XmAb 2+1 GPC3 x CD3 bispecific antibodies:

- Are humanized, well-behaved, and efficiently purified and manufactured.
- Selectively recruit T cells in vitro to kill high-expressing GPC3+ cancer cells, while avoiding cytotoxicity to off-target proxy cell line
- Match the efficacy of 1st gen T cell engagers in 2D and 3D longitudinal models in vitro

These results support clinical testing of a 2+1 GPC3 x CD3 bispecific antibody as a potential therapeutic option for patients with HCC and subsets of other GPC3+ indications.

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