

BIOEN 570

A Proposal for Using CAR T-Cell AND Gates with MUC1 and MSLN Receptors to Treat Pancreatic Adenocarcinoma

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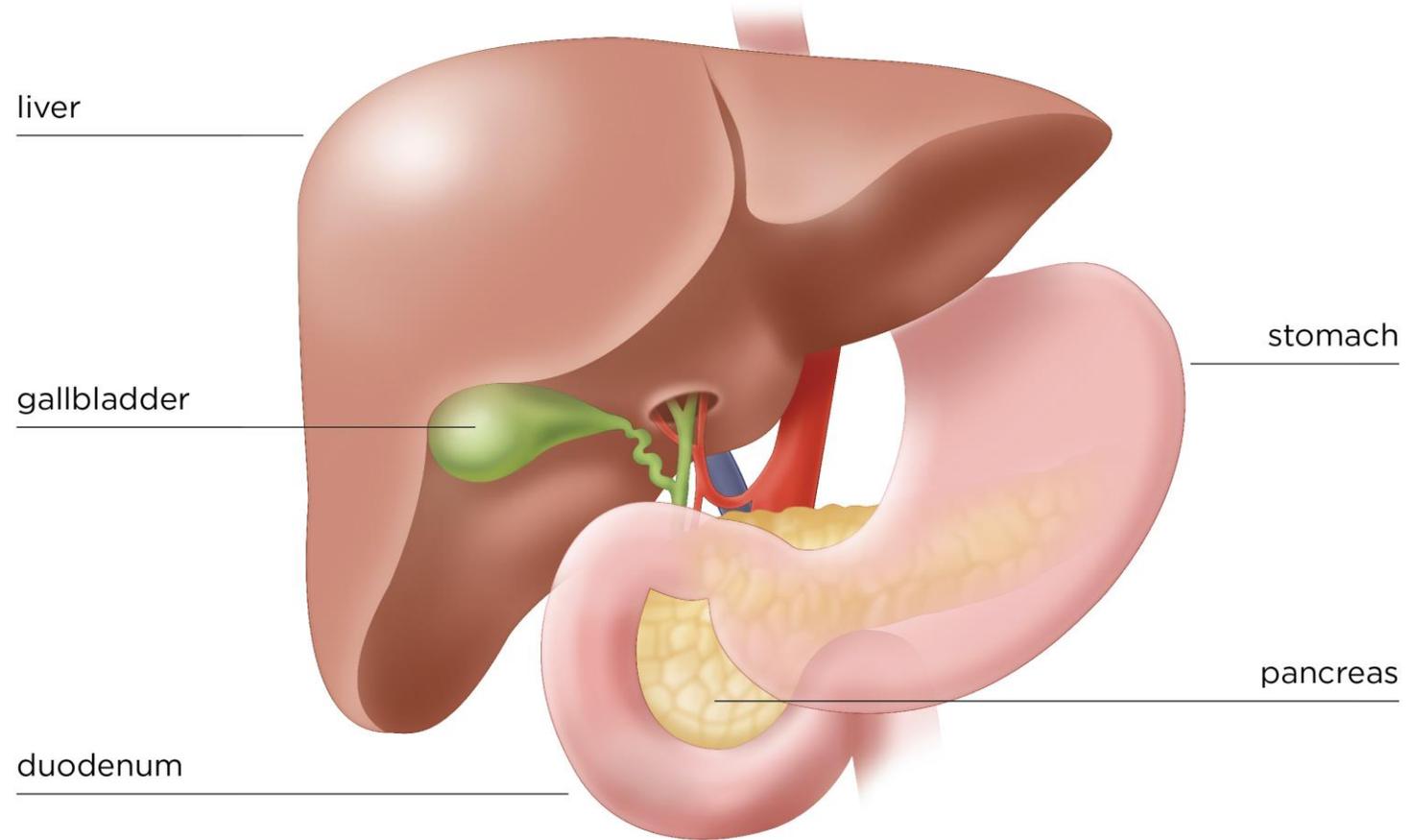
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‘Don’t mess with the pancreas’

Surgery has three rules.

- Eat when you can
- Sleep when you can
- Don’t mess with the pancreas

‘...it's a poorly-encapsulated organ that is a slurry of cells and enzymes that are trying to digest everything they can inside the body, is **intimately involved with the superior mesenteric artery and vein, the celiac plexus, overlies the aorta**, has a ductal system that must remain intact, is prone to form high-output fistulas, can trigger fatal systemic inflammation and — *and*— is so anatomically crucial that **removal of the head of the pancreas can only be accomplished if you also remove part of the duodenum, gallbladder, part of the biliary tract and half the stomach.**’

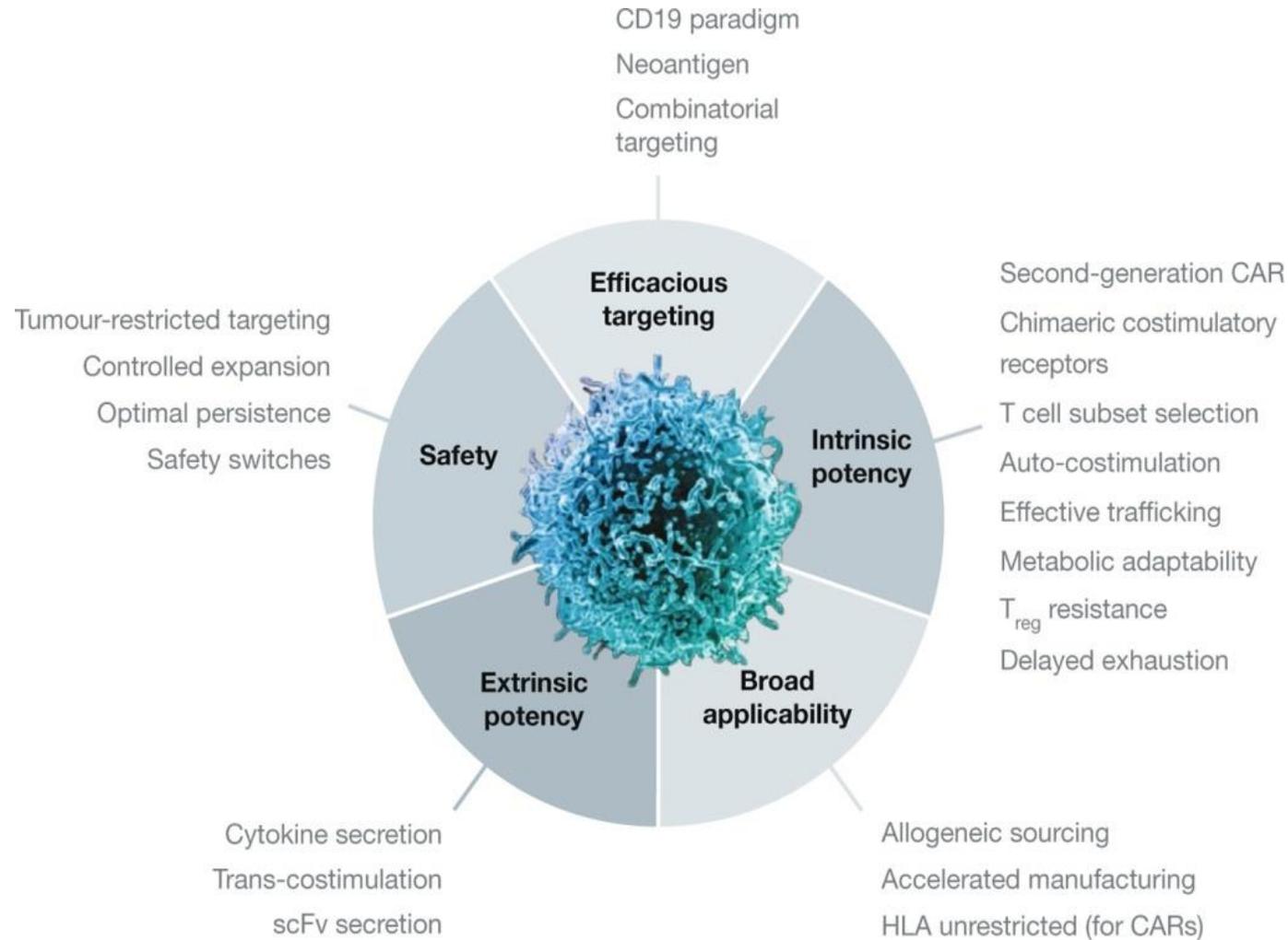


Why Pancreatic Cancer?

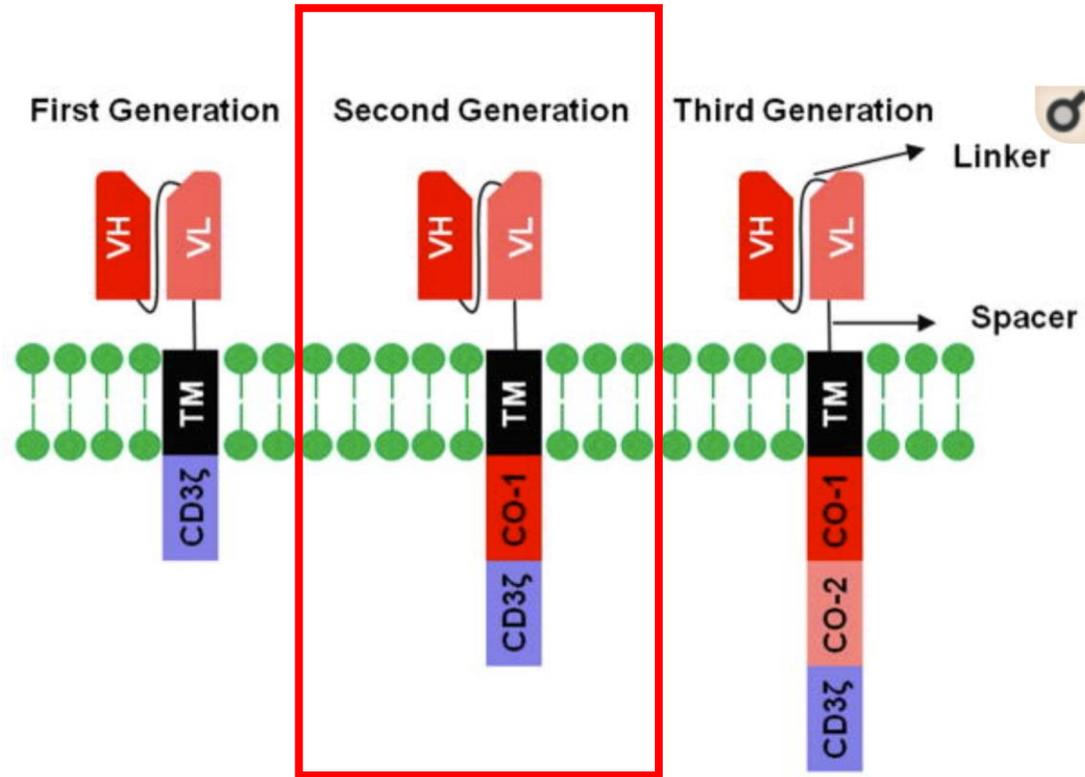
Pancreatic
Cancer
UK



Therapeutic T cell design: goals and strategies

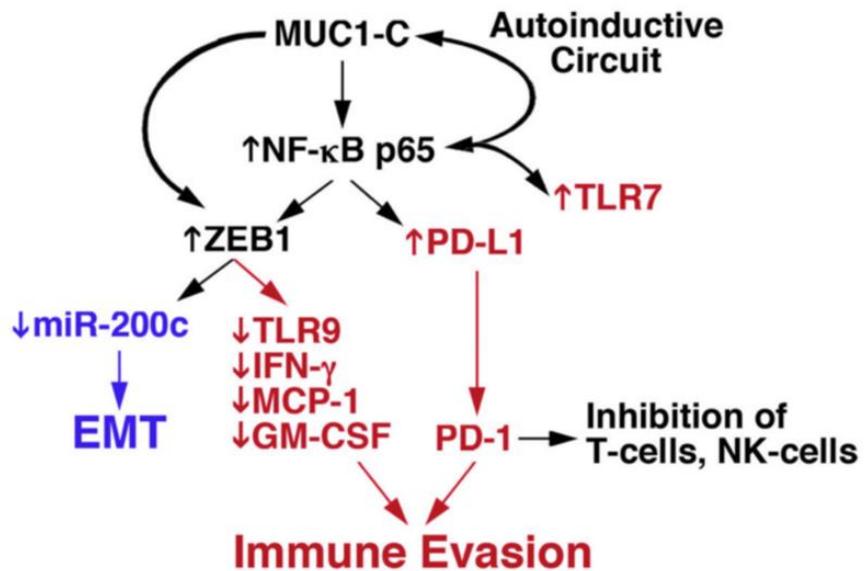


Type of Chimeric Antigen Receptors (CARs)

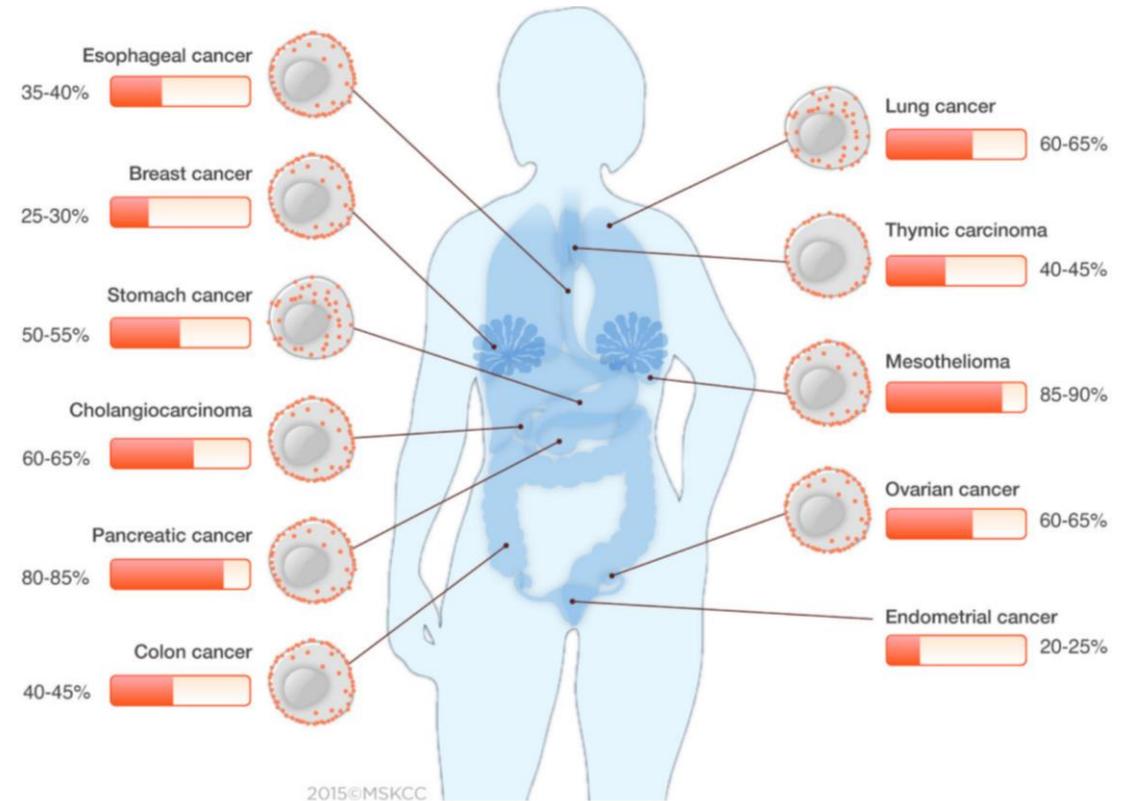


Structure of chimeric antigen receptors (CAR). CARs are composed of a single-chain fragment variable (scFv, containing the heavy chain variable domain (VH) and the light chain variable domain (VL) of a monoclonal antibody attached together via a flexible linker) linked via a spacer sequence to a transmembrane (TM) domain and to the CD3 ζ chain (first-generation CARs). Second- and third-generation CARs additionally contain one or two costimulatory domains, respectively. VH, heavy chain variable domain; VL, light chain variable domain; TM, transmembrane domain; co-1, costimulatory domain 1; co-2, costimulatory domain 2.

Target antigens: MUC1 and MSLN



Mechanism of immune evasion triggered by MUC-1 overexpression

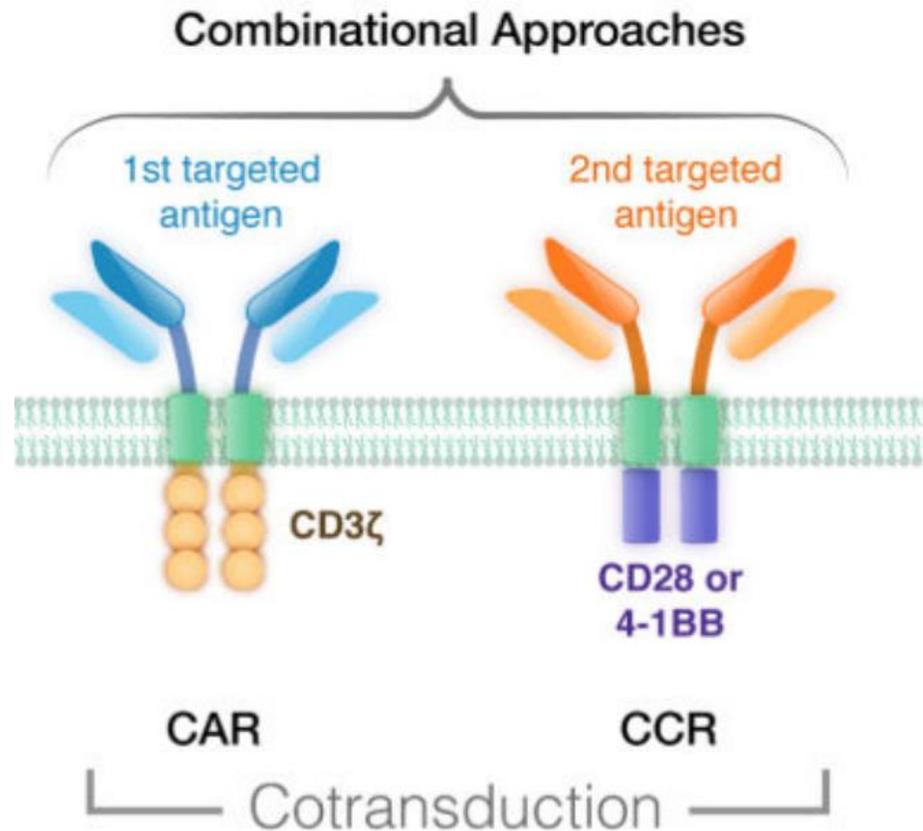


Frequency and distribution of MSLN in solid tumors

MSLN and MUC1 expressed highly in pancreatic cancer, with several clinical trials

Mesothelin	PDA (up to 100%), MPM (85%), Ovarian (70%), lung adenocarcinoma (53%, advanced; 69%, early stage), GBM	-	NCT02930993	I
		?	NCT02959151	I/II
		PD-1/TCR KO	NCT03545815	I
		α CTLA-4/PD-1	NCT03182803	I/II
		-	NCT01583686	I/II
		α PD-1	NCT03030001	I/II
		PD-1 KO	NCT03747965	I
		-	NCT03198052	I
		α PD-1	NCT03615313	I/II
		-	NCT03267173	Early I
		-	NCT03356795	I/II
		BB ζ	NCT02792114	N/A
		28 ζ	NCT02414269	I
		MCY-M11	NCT03608618	I
		MUC1 (mucin 1) (17)	HCC, NSCLC, pancreatic, breast, glioma, colorectal, gastric	α CTLA-4/PD-1
-	NCT02587689			I/II
\pm PD-1 KO T cells	NCT03706326			I/II
\pm PD-1 KO T cells	NCT03525782			I/II
-	NCT03198052			I
-	NCT03267173			Early I
-	NCT03356795			I/II
4SCAR-IgT	NCT03356782			I/II
-	NCT03633773			I/II
-				
MUC16 ecto (mucin 16) (18, 53)	Ovarian	TCR-directed	Clinical	
		CAR	Preclinical	

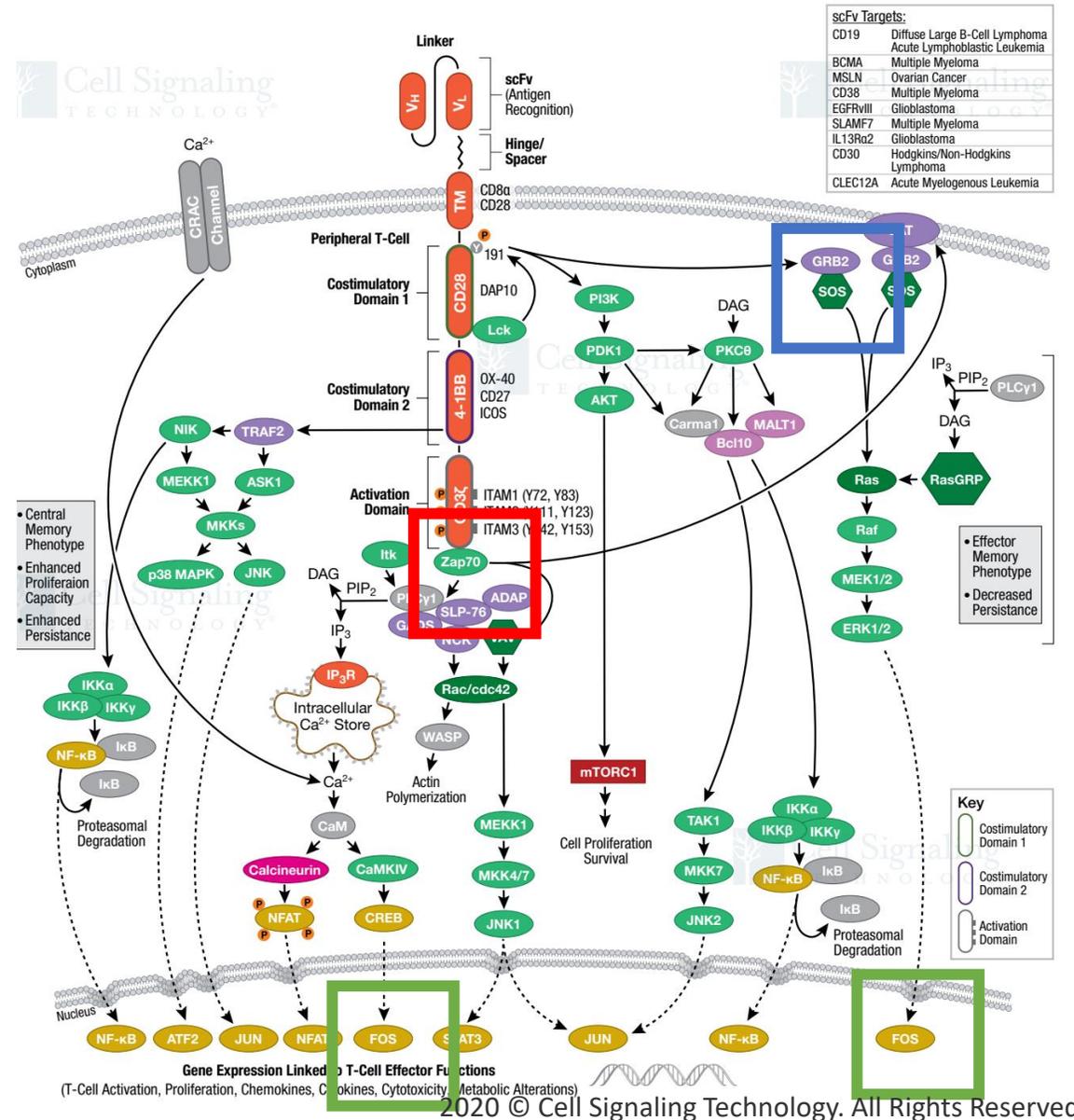
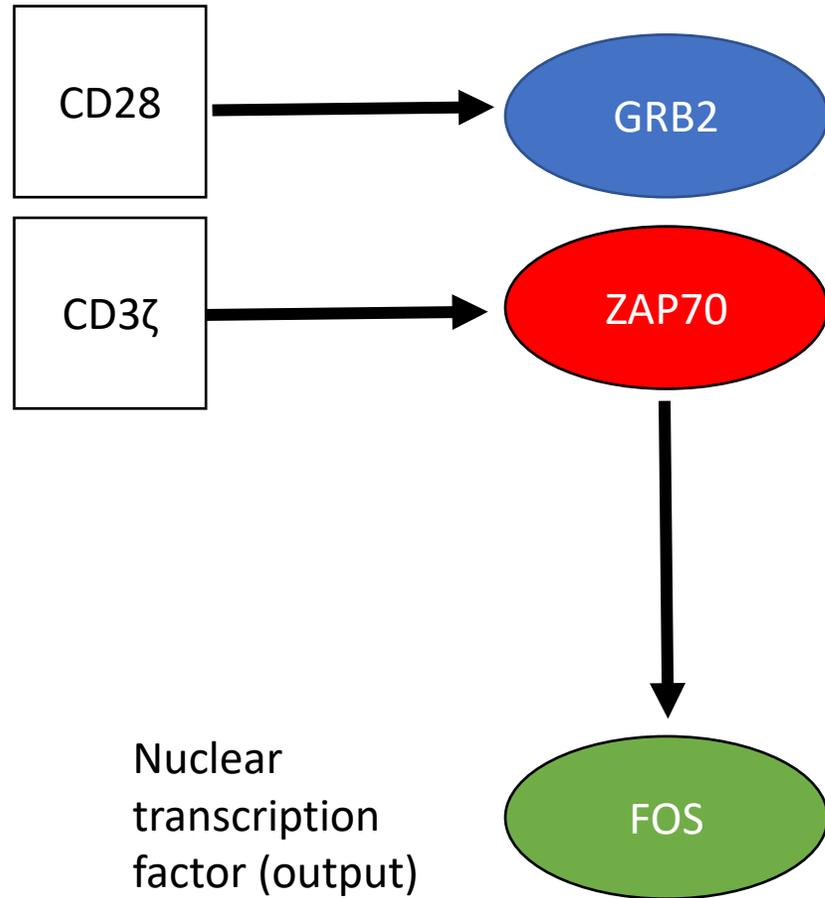
CAR design



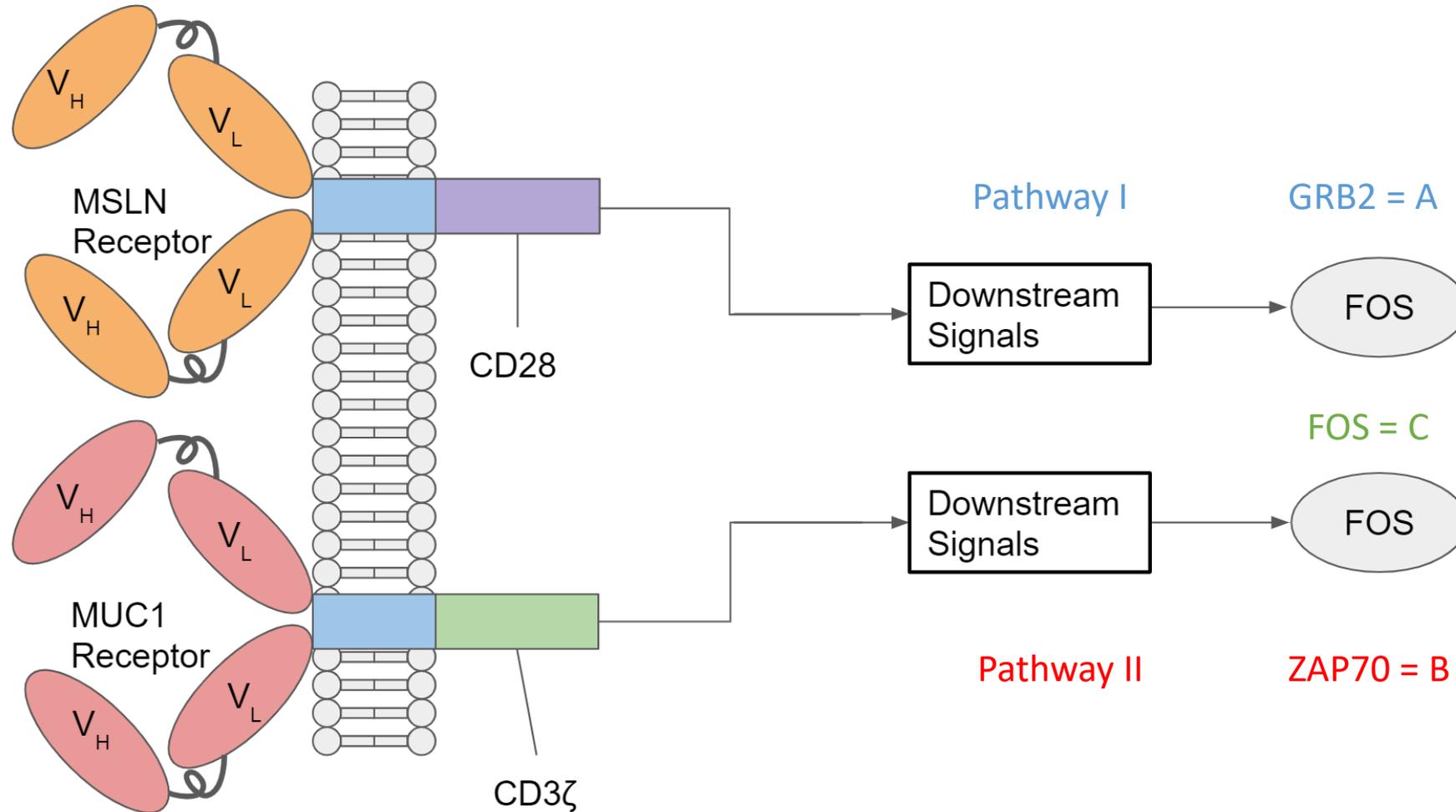
Benefits of combinatorial approach:

- Reducing incidences of T cell anergy
- Reducing on-target/off tumor toxicity effects by precision targeting of cancer cells

Target molecules and pathways to model



Synthetic Circuit Design

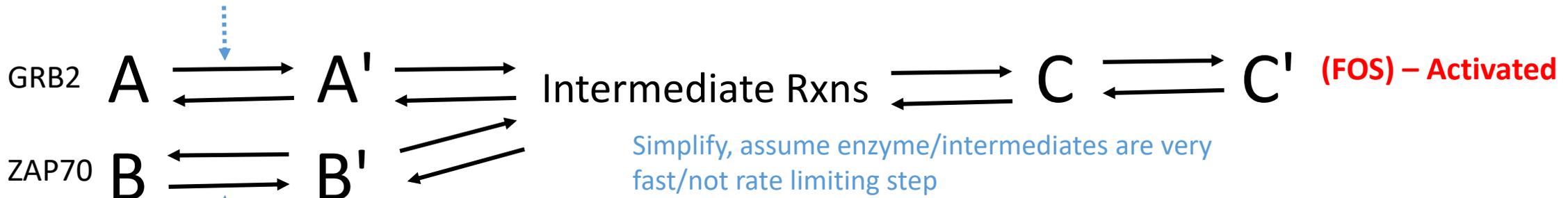


Assumptions:

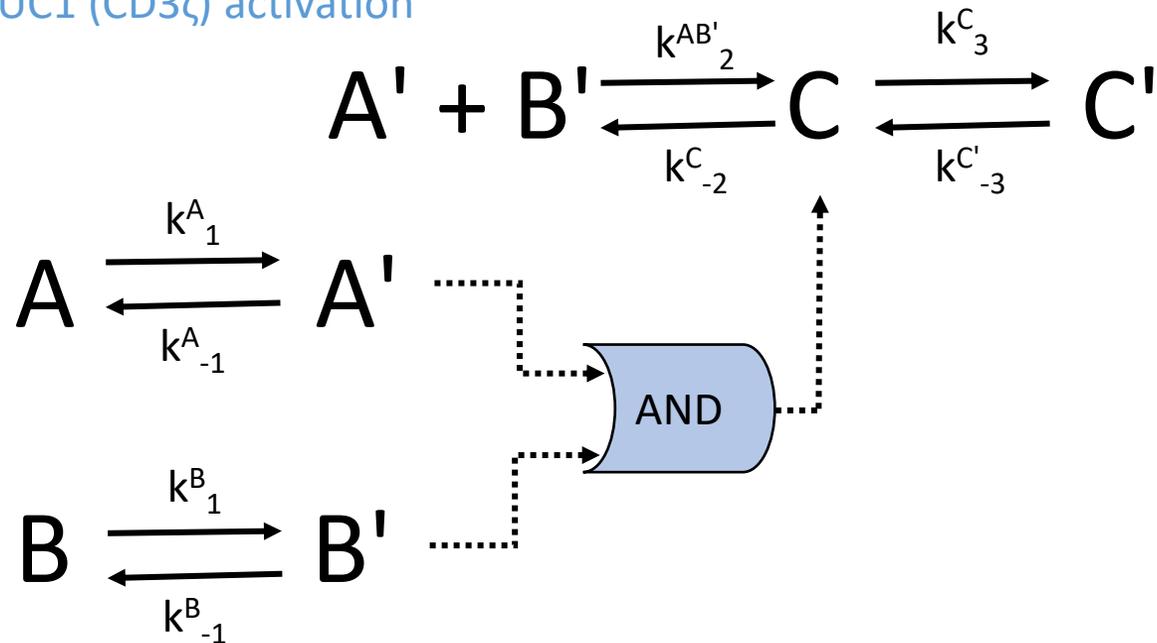
- Only GRB2₀ and ZAP70₀ (A and B) are present initially
- First order reactions
- Very fast intermediate reactions
- Constant volume, biological temperature, pressure
- $k_{1}^A = 3$ (1/nmol*s) $k_{-1}^A = 1$ (1/s) $k_{2}^{AB} = 7$ (1/nmol*s)
 $k_{-2}^{AB} = 2$ (1/s) $k_{1}^B = 5$ (1/nmol*s) $k_{-1}^B = 2$ (1/s)
 $k_{3}^C = 20$ (1/nmol*s) $k_{-3}^C = 20$ (1/s)
- A = 1, A' = 0, B = 2, B' = 0, C = 0, C' = 0 (nmol)

Mathematical Modeling

MSLN (CD28) activation



MUC1 (CD37) activation



$$dA'/dt = k^A_1 A - k^A_{-1} A' + k^C_{-2} C - k^{AB'}_2 A' B'$$

$$dB'/dt = k^B_1 B - k^B_{-1} B' + k^C_{-2} C - k^{AB'}_2 A' B'$$

$$dC'/dt = k^C_3 C - k^{C'}_{-3} C'$$

$$dA/dt = -k^A_1 A + k^A_{-1} A'$$

$$dB/dt = -k^B_1 B + k^B_{-1} B'$$

$$dC/dt = -k^C_3 C + k^{C'}_{-3} C' + k^{AB'}_2 A' B' - k^C_{-2} C$$

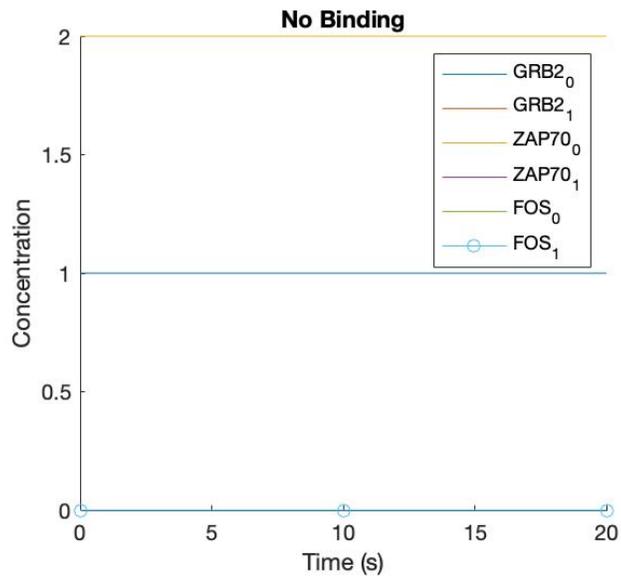


Fig. 1

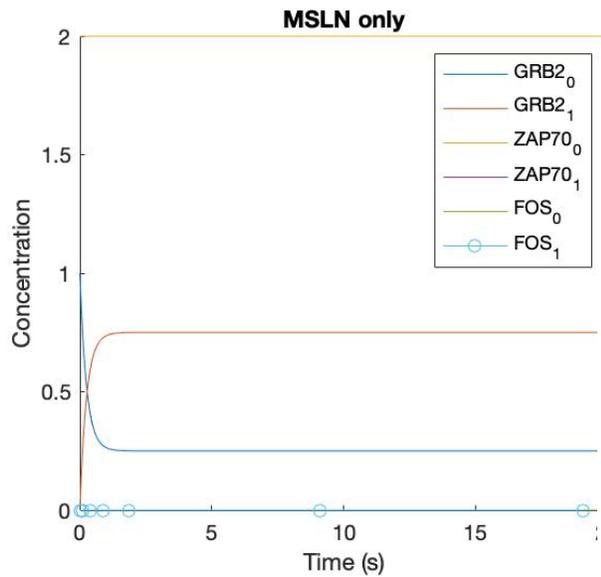


Fig.2

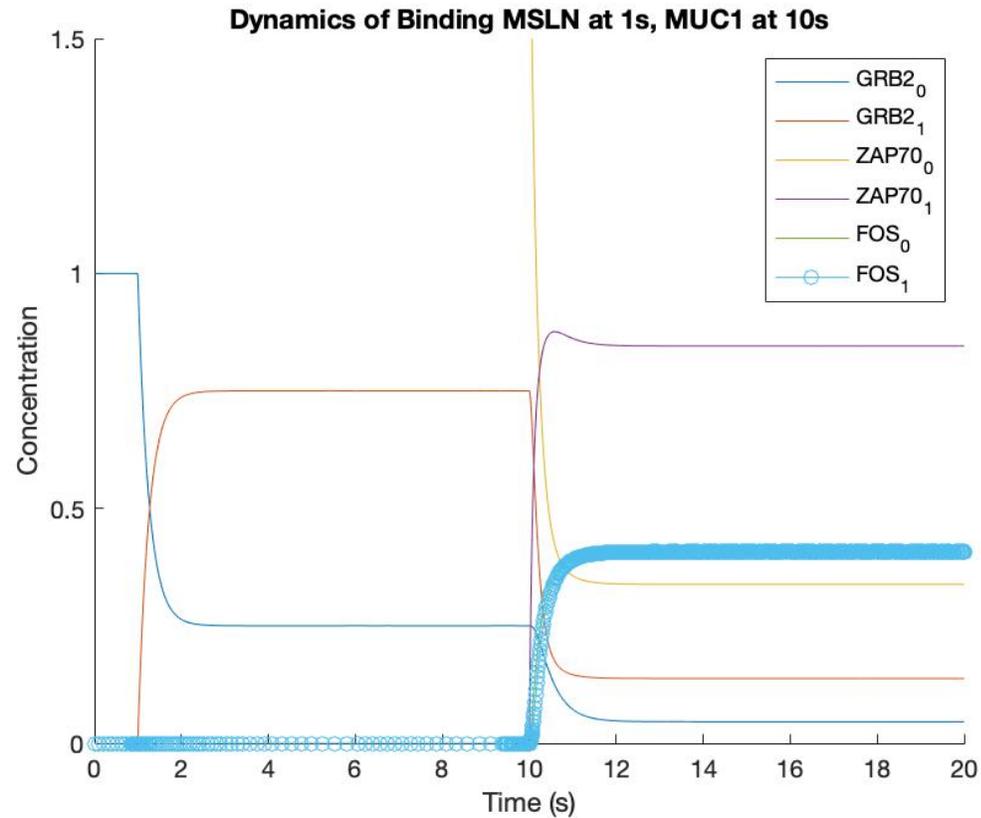


Fig. 5

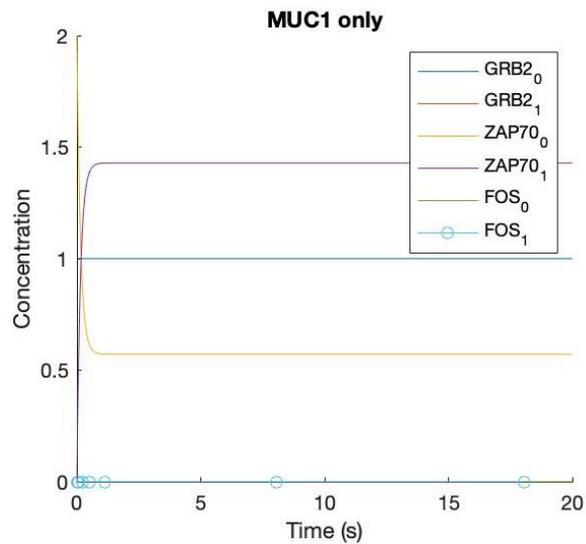


Fig. 3

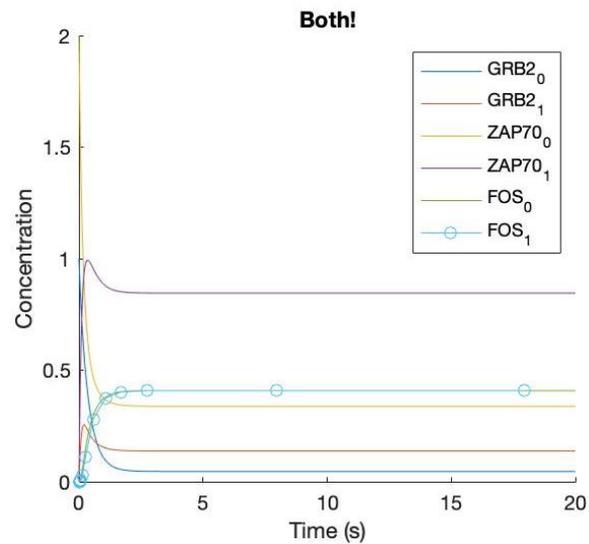
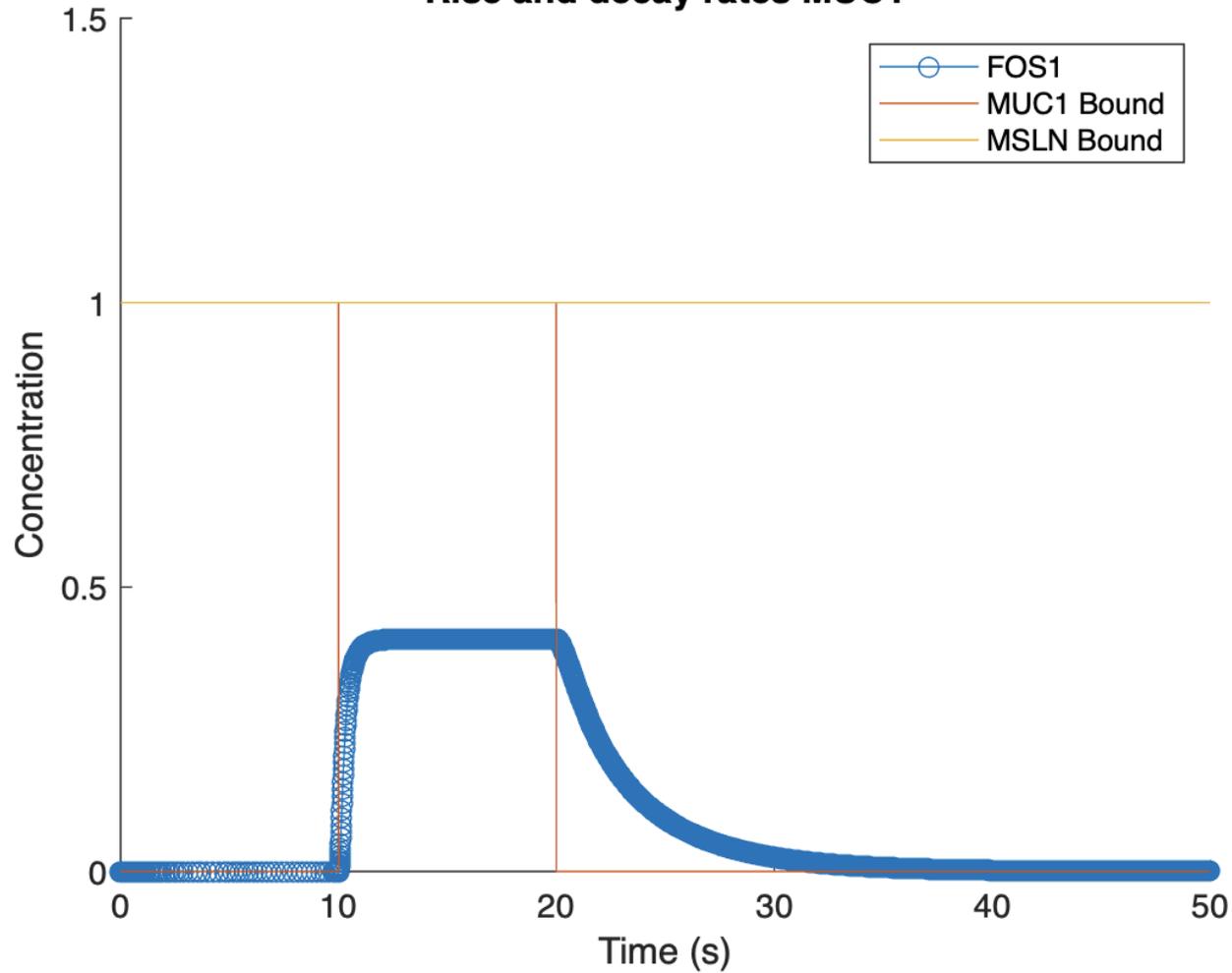


Fig.4

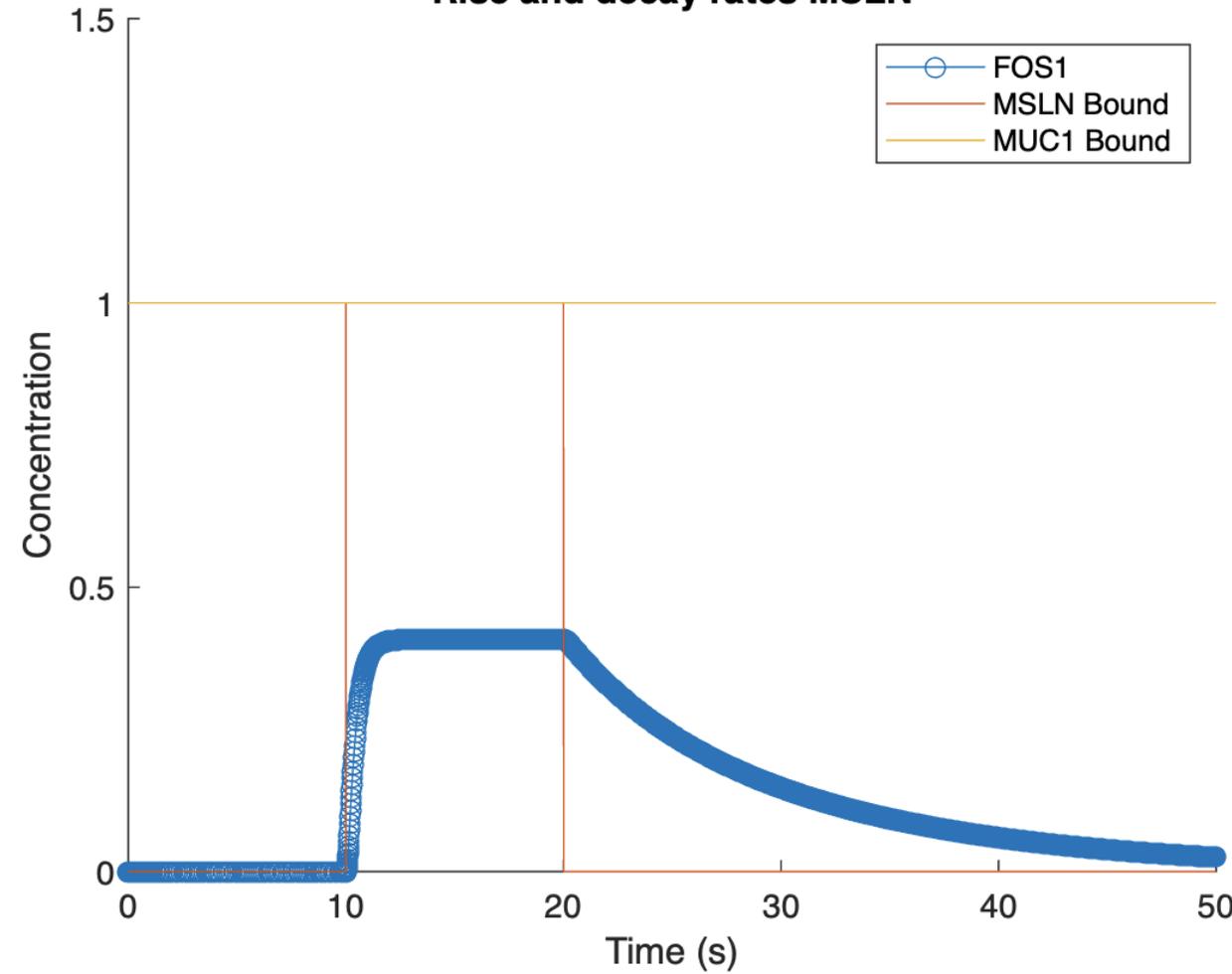
The **AND** gate works!

Kinetics – Fast!

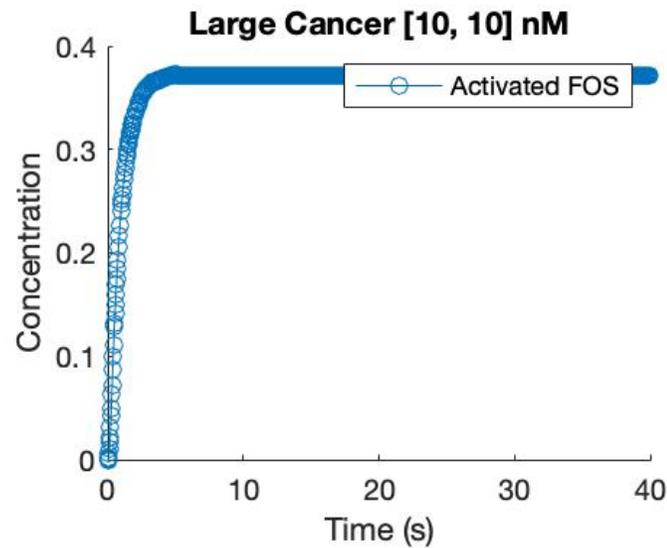
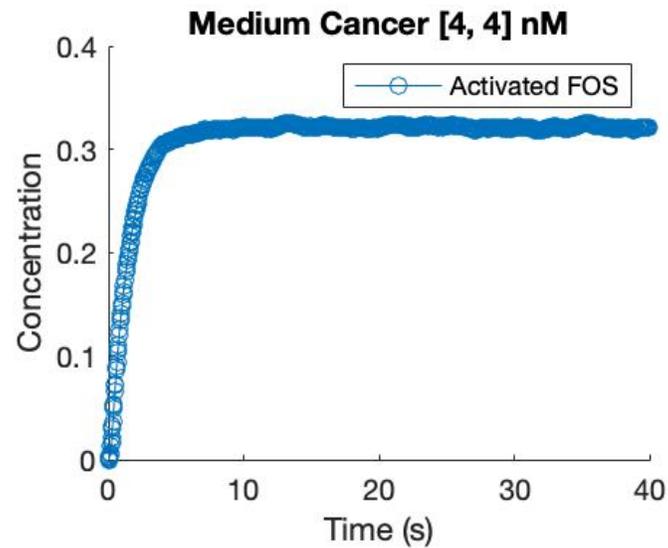
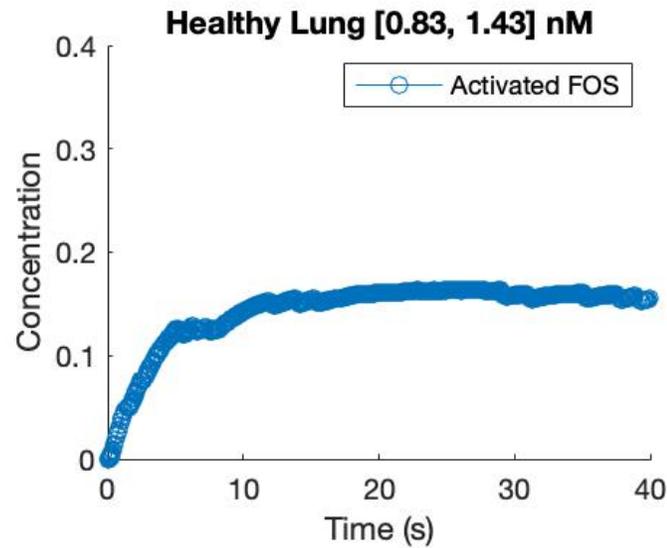
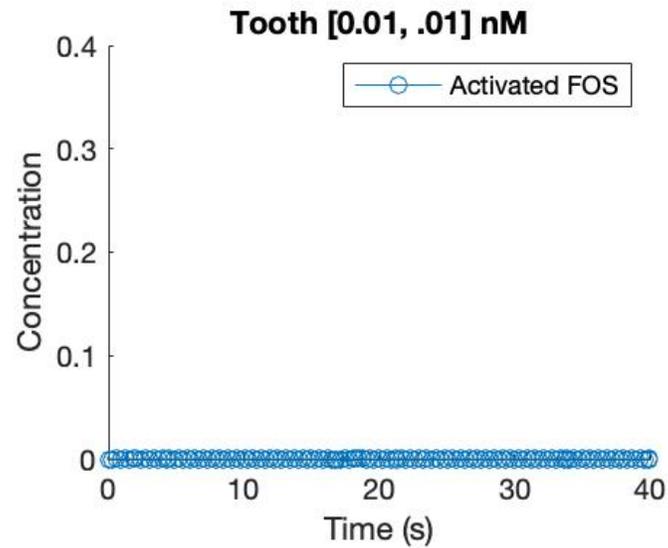
Rise and decay rates MUC1



Rise and decay rates MSLN



Activity in Tissues



Clear difference between healthy tissues and cancer!

Summary

- The **AND** gate works!
- Activation and deactivation is FAST!
- Has the potential to be selective for tumors