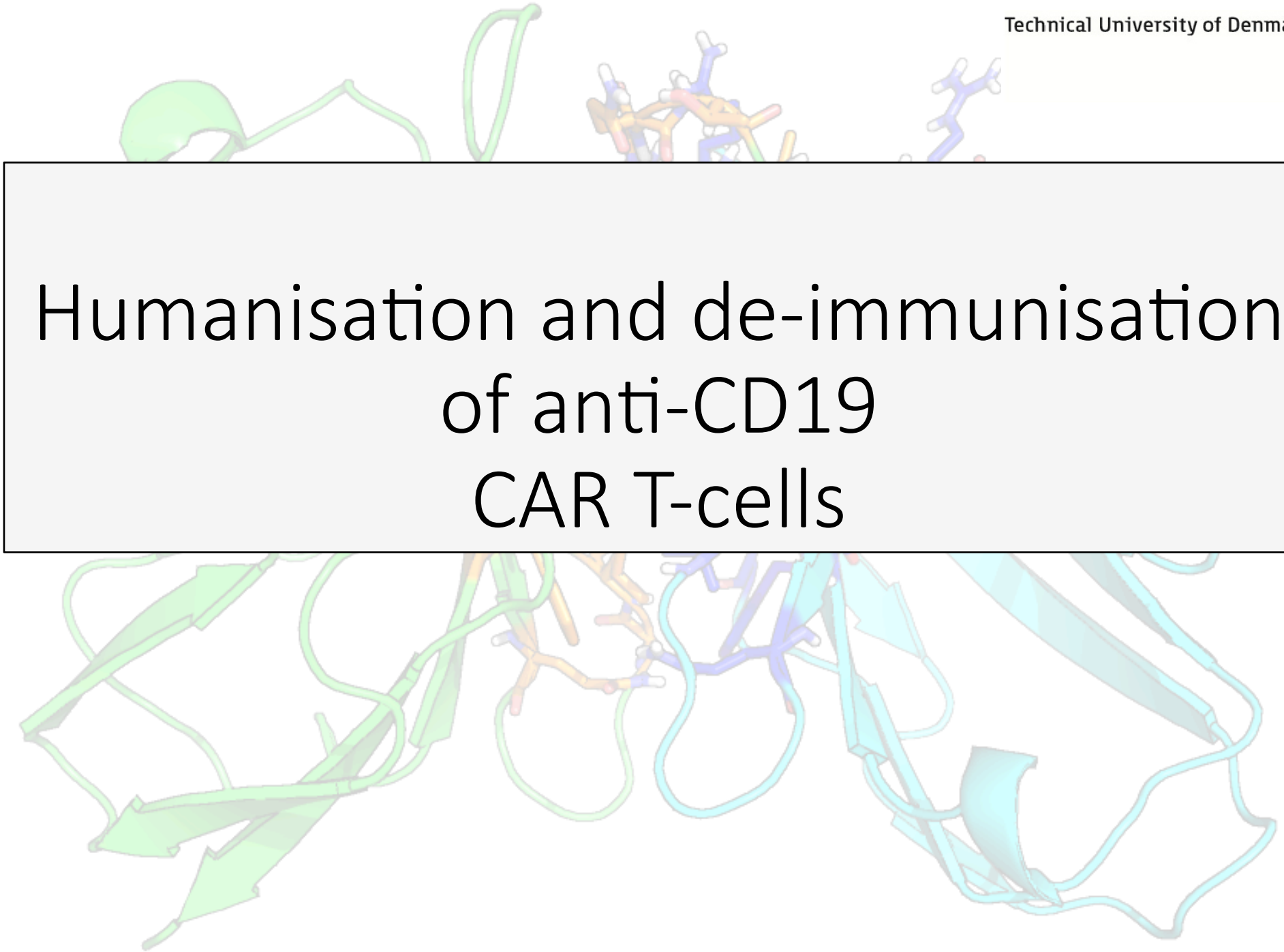
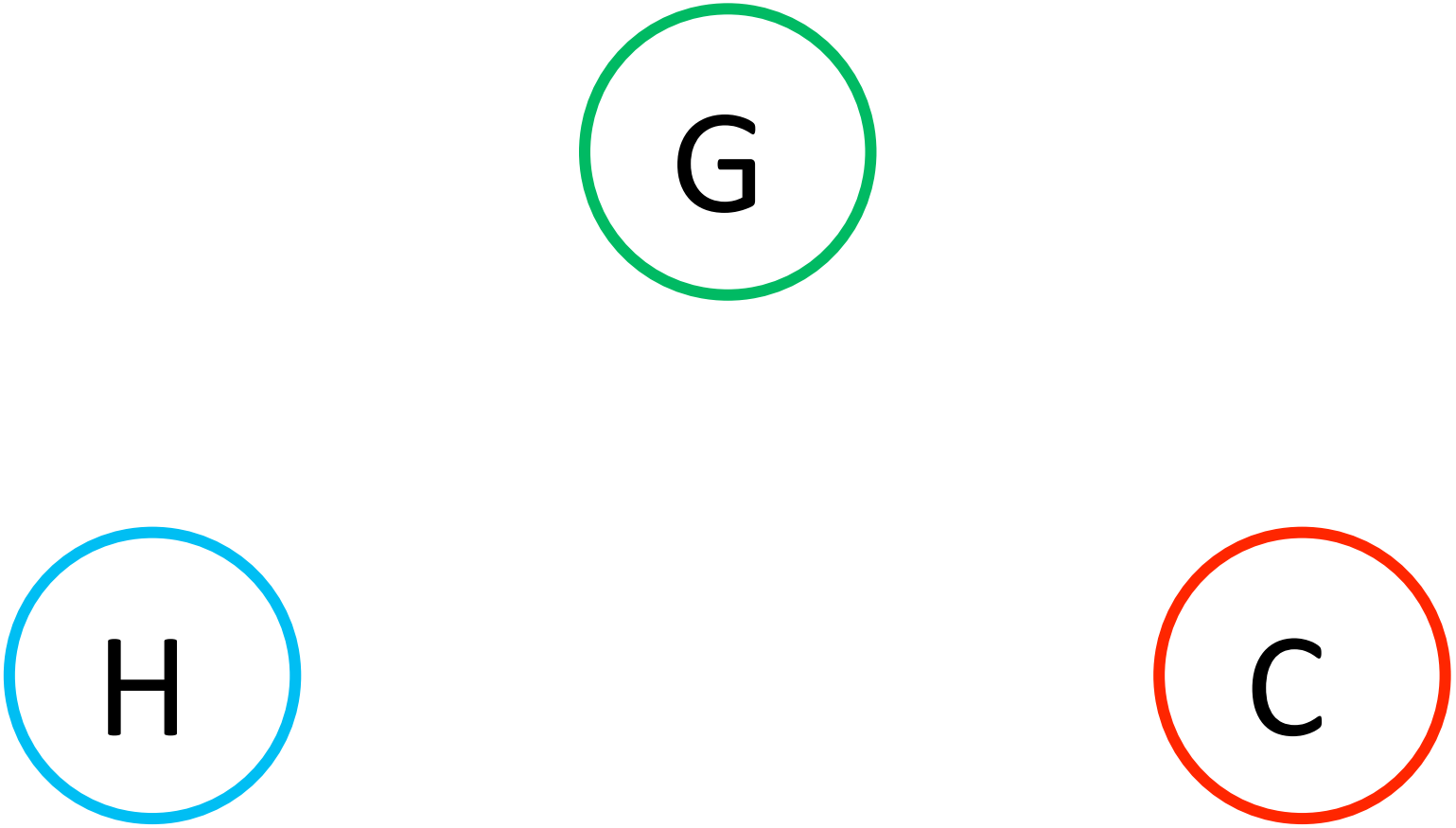
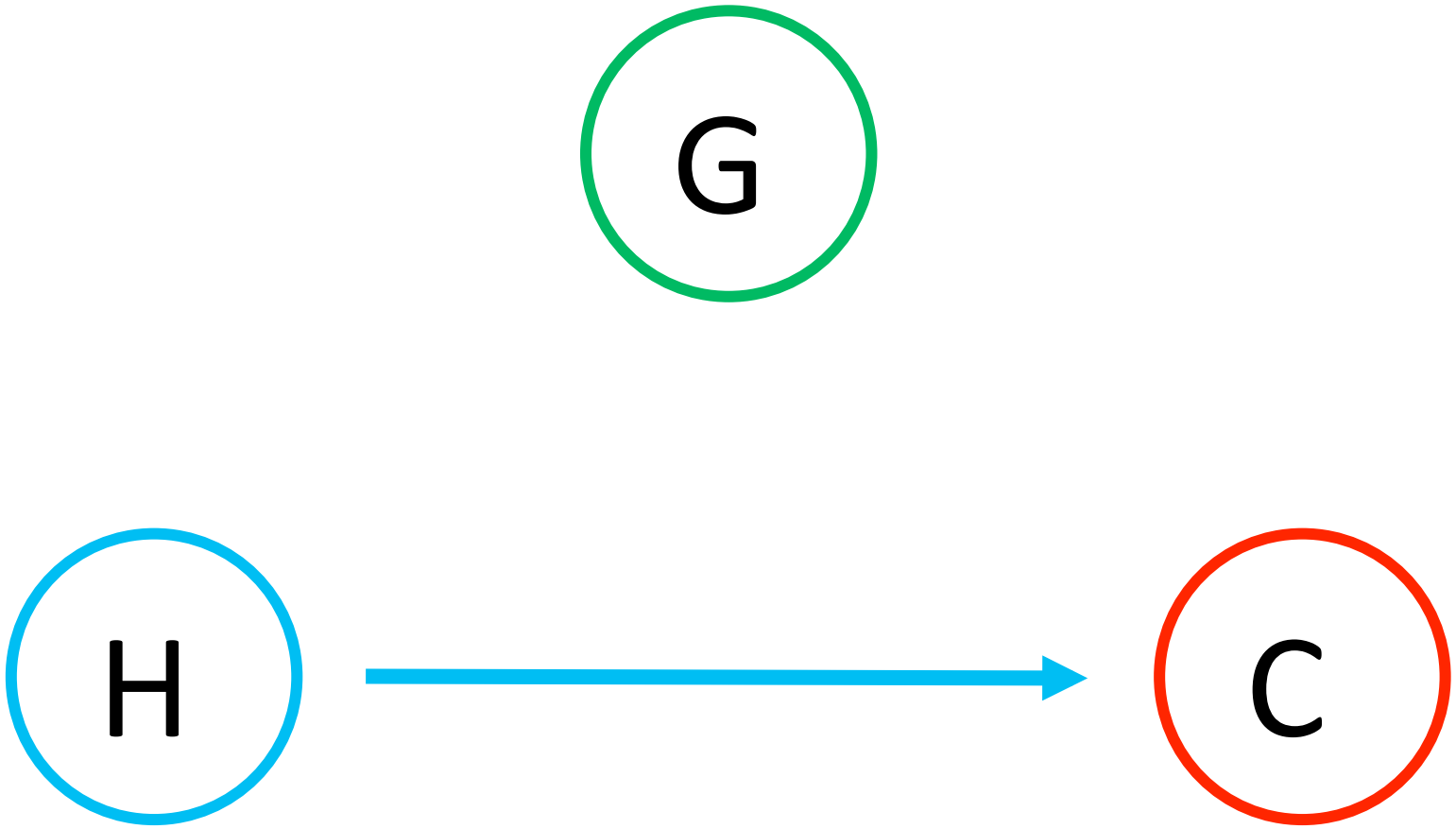
Humanisation and de-immunisation of anti-CD19 CAR T-cells



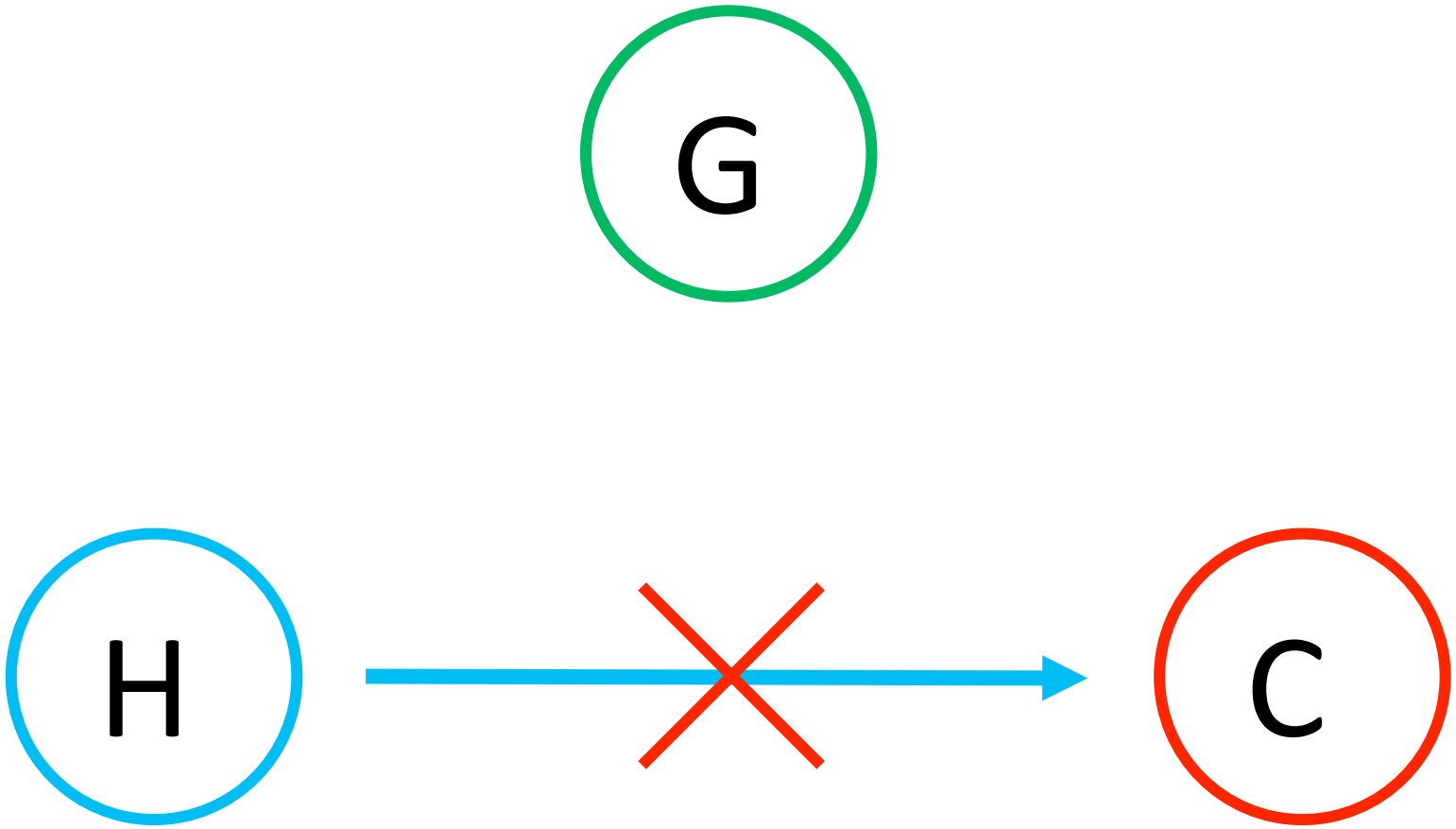
Host, graft, and cancer



Host, graft, and cancer



Host, graft, and cancer



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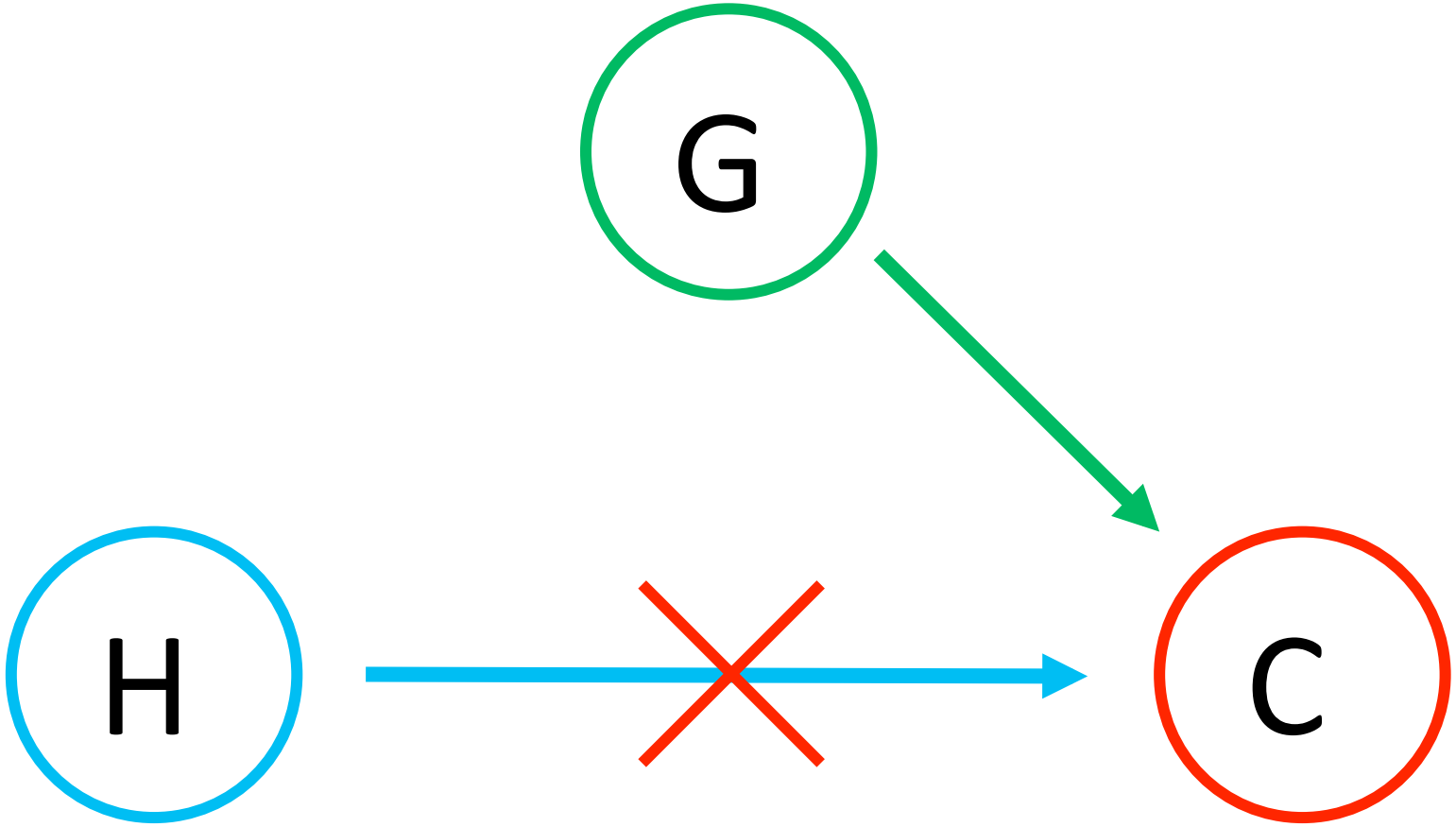
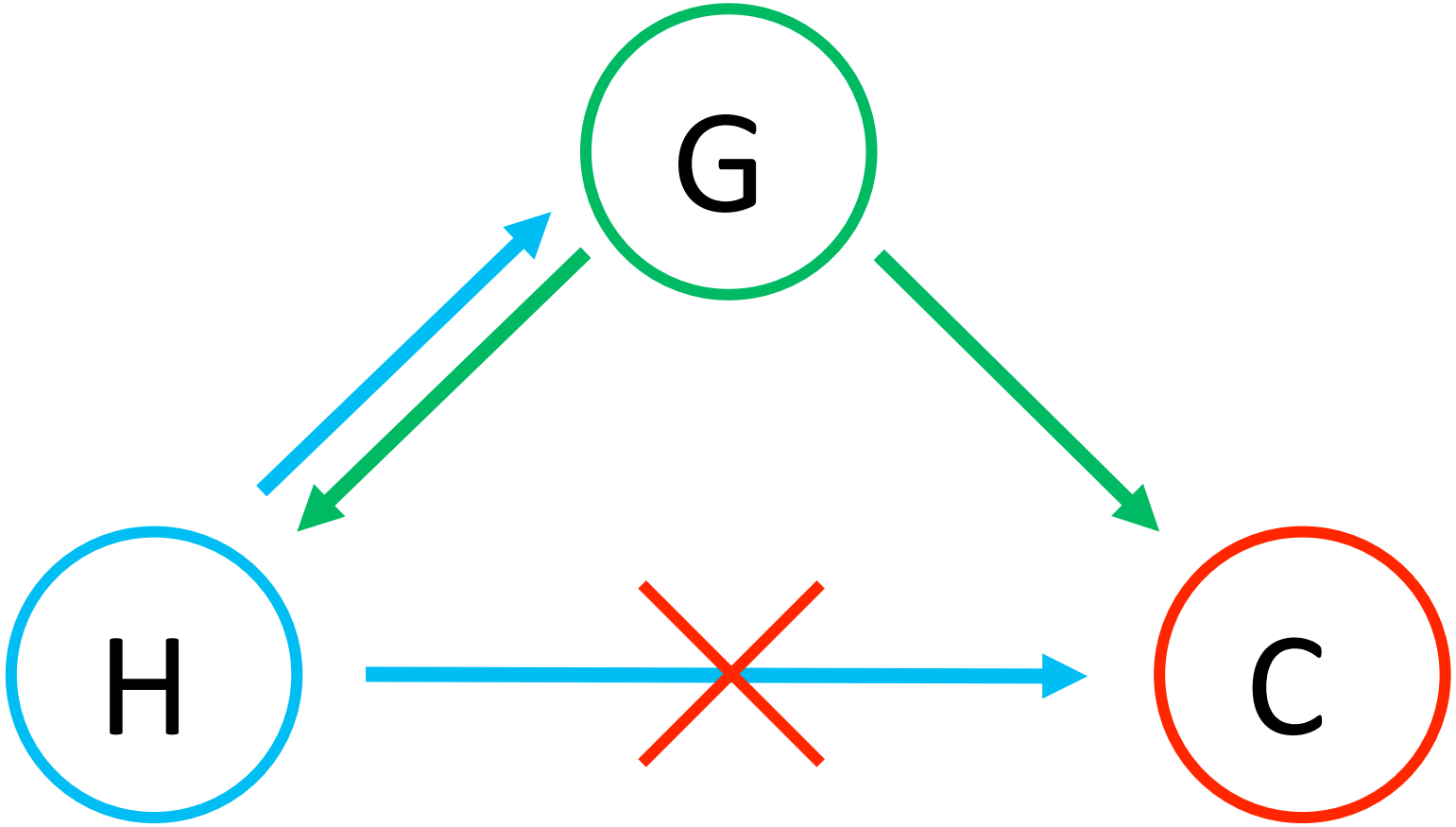


Host, graft, and cancer

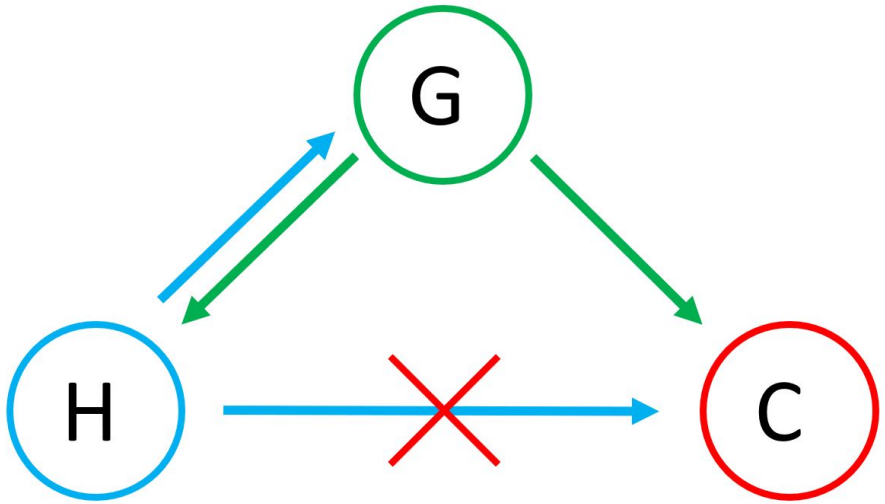


Immunotherapy and aim

- Immunotherapy for leukemia
- Focus on T lymphocyte (T-cell) immunotherapies:

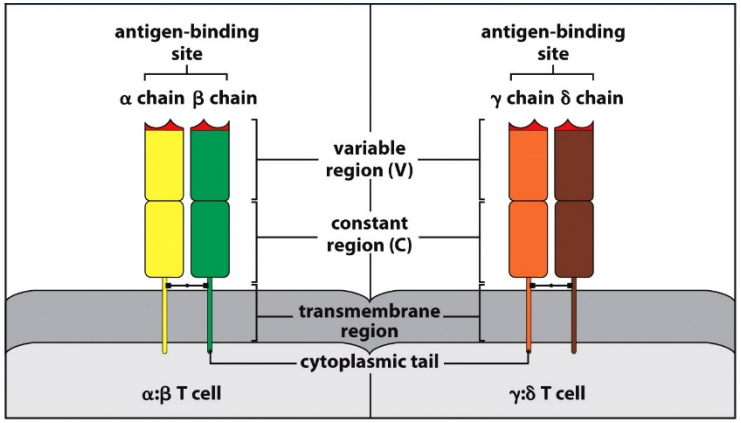
- Targeting leukemia
  - αβ **TCR** recognizing the SLL antigen of the PRAME gene
  - **CAR** recognizing CD19

- Aim: propose solutions to overcome side effects

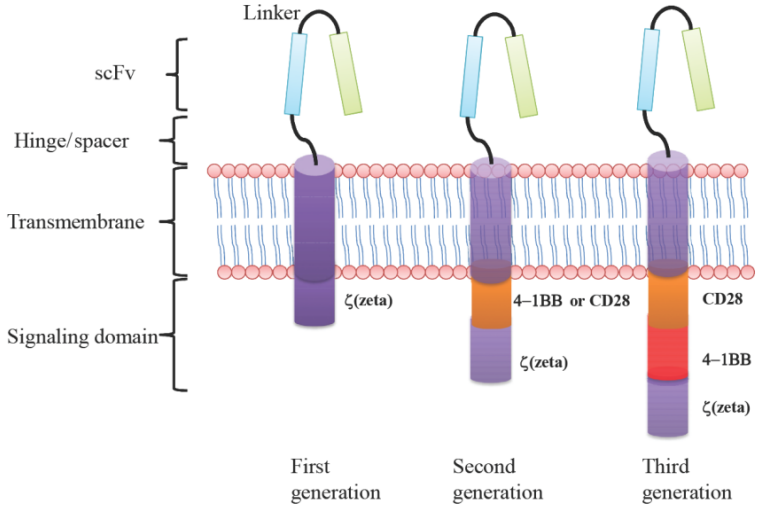


Lymphocyte molecules

- T-cell receptors (TCRs)
- Chimeric antigen receptors (CARs)

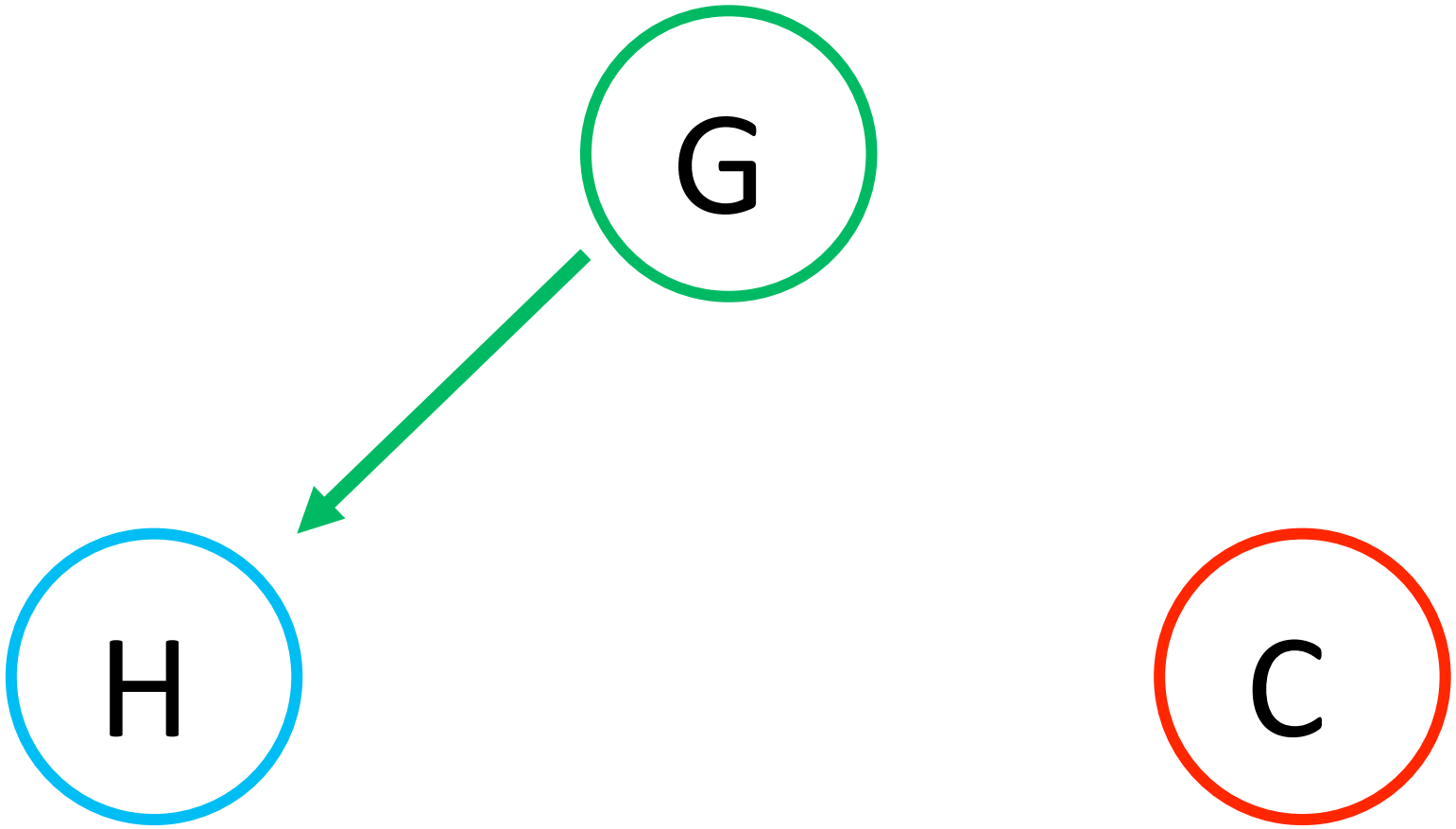


Parham et al. 2009



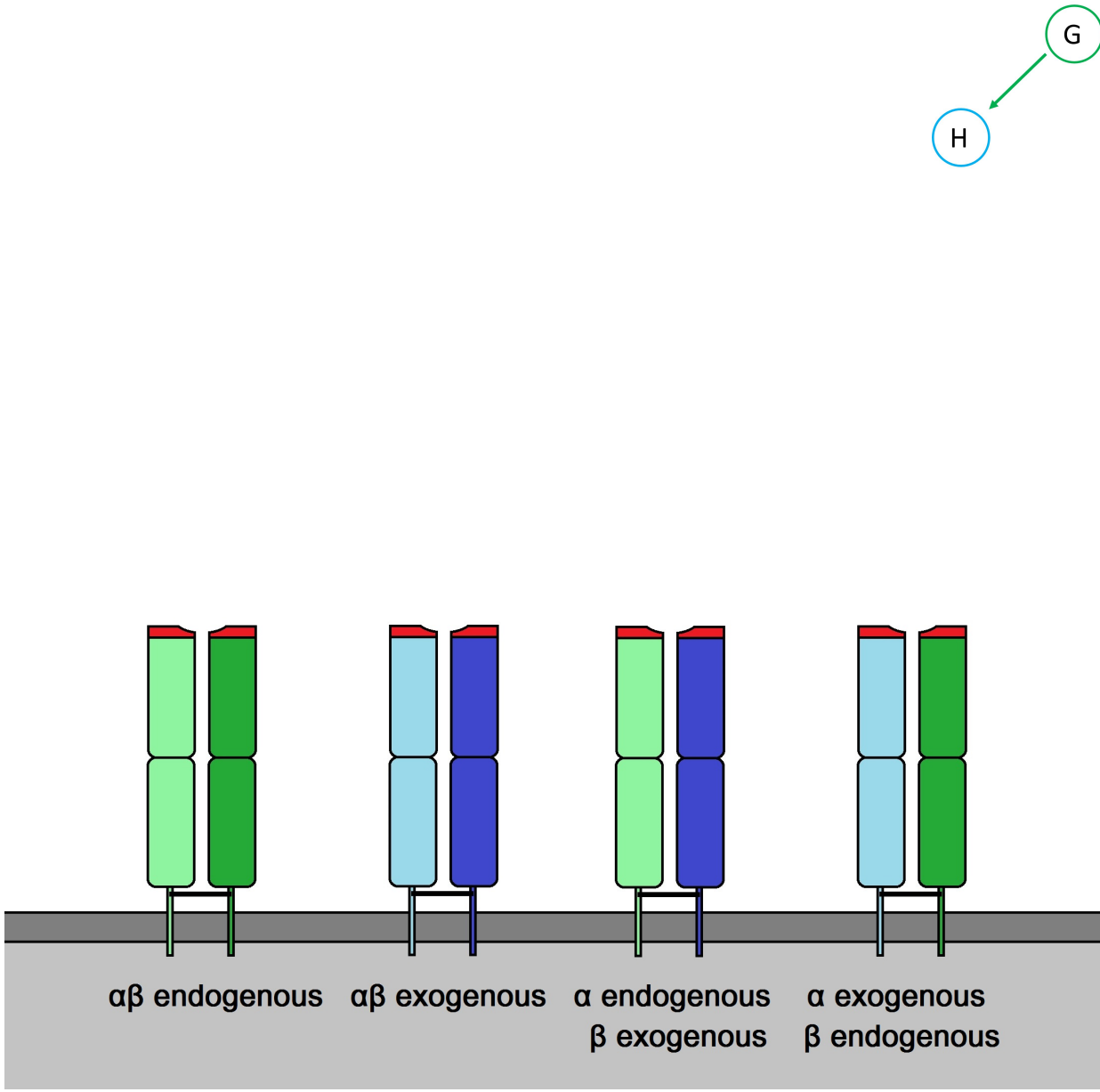
Dai et al. 2016

Graft versus host



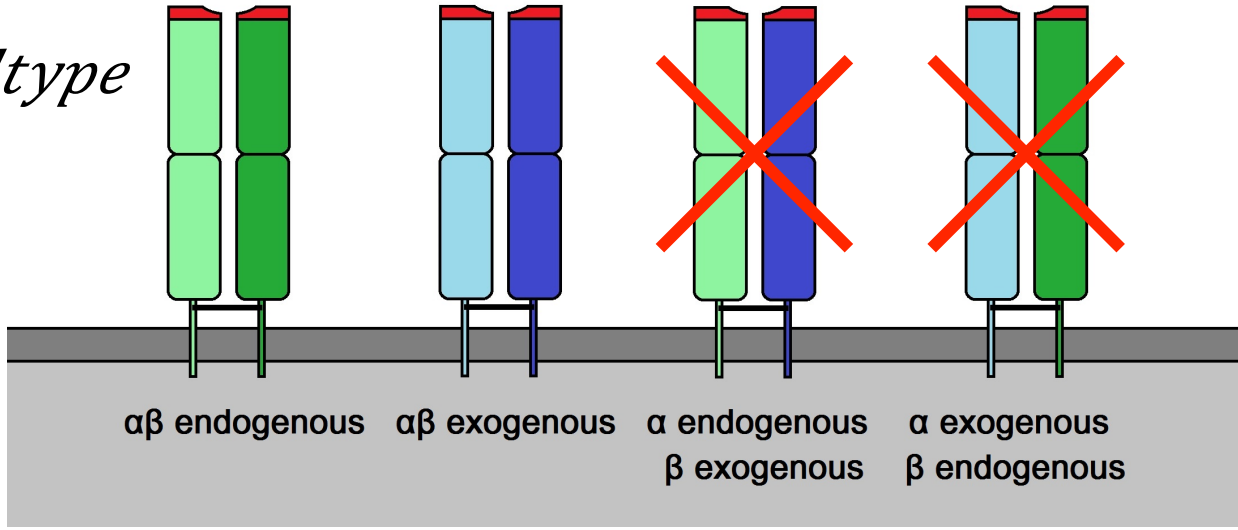
Graft versus host

- TCR immunotherapy
- Transfusion
  - Endogenous TCR complex
  - Exogenous TCR complex
  - Expression of 4 complexes
  - Mispaired unfavorable
- Our aim: avoid mispaired TCR complexes



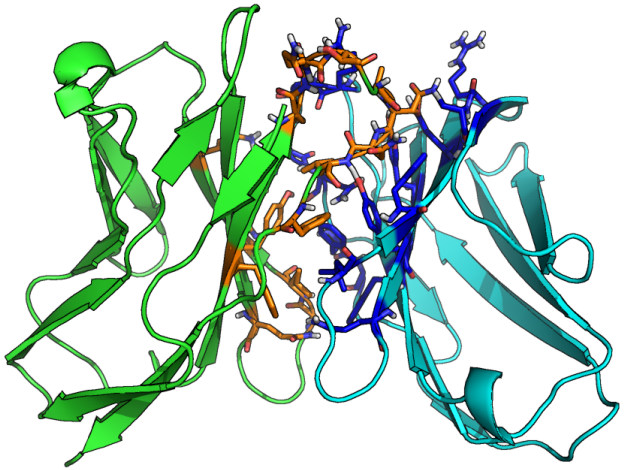
Protein stability and thermodynamics

- Stabilizing and destabilizing energies
  - Affected by each amino acid
- Protein thermodynamics
  - ΔG = free energy of stability
  - $\Delta\Delta G = \Delta G{\downarrow}mutant - \Delta G{\downarrow}wildtype$
  - $\Delta\Delta G > 0$, destabilizing
  - $\Delta\Delta G < 0$, stabilizing

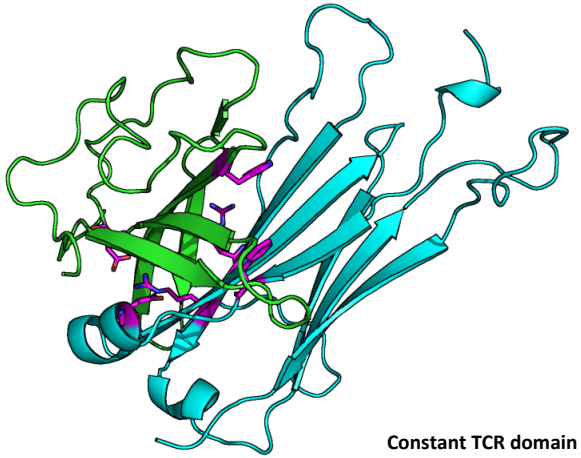


Interface analysis

- Paired mutations
  - Destabilize mispaired complexes
  - Stabilize exogenous complex
- Three criteria for mutations:
  1) $\Delta G{\downarrow}WT < \Delta G{\downarrow}\alpha'\beta$
     $\Delta G{\downarrow}WT < \Delta G{\downarrow}\alpha\beta'$
  2) $\Delta G{\downarrow}WT \gtrsim \Delta G{\downarrow}\alpha'\beta'$
  3) $G{\downarrow}WT(\alpha) \approx G{\downarrow}\alpha'$
     $G{\downarrow}WT(\beta) \approx G{\downarrow}\beta'$
- Energy prediction: FoldX and Rosetta

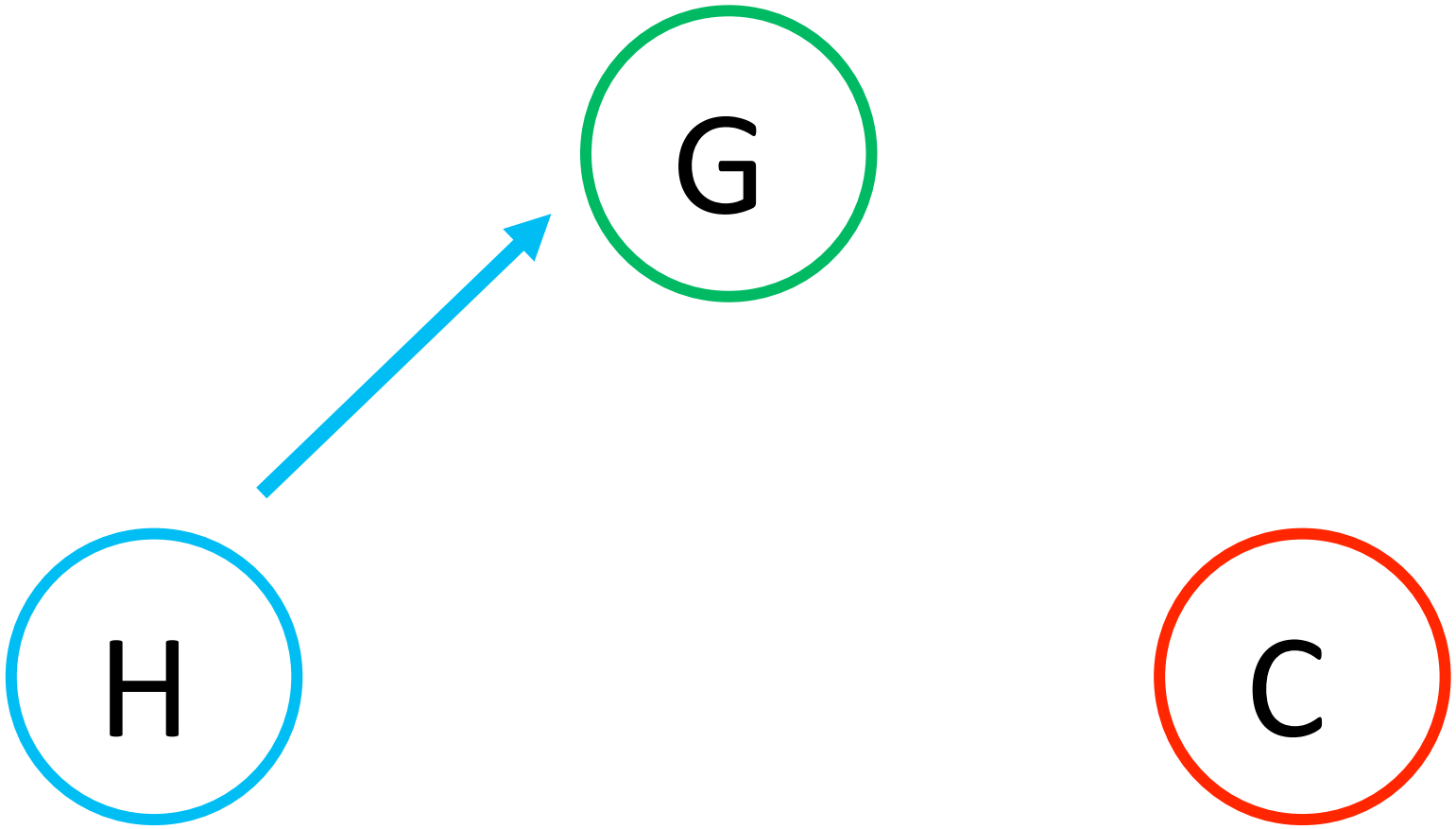


Variable TCR domain



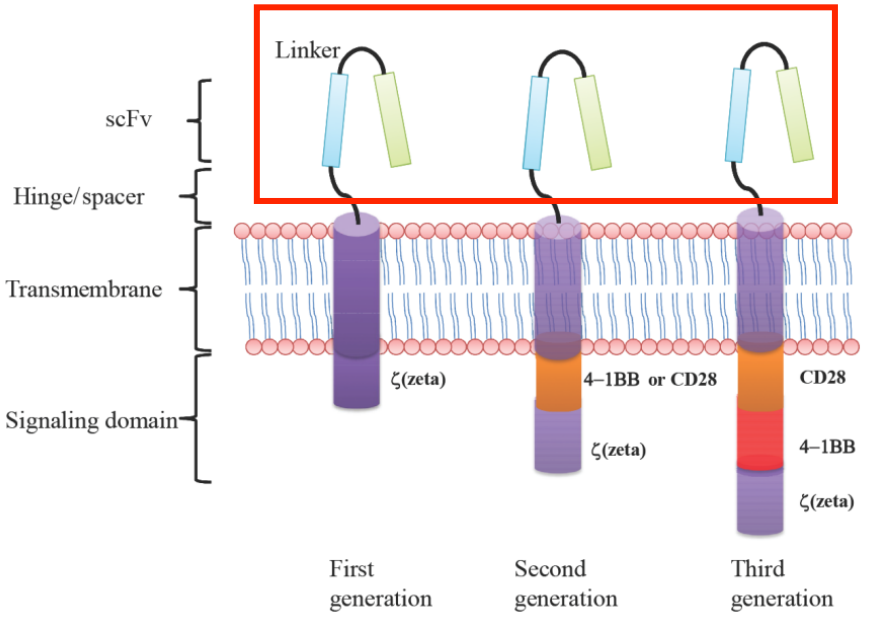
Constant TCR domain

Host versus graft



Host versus graft

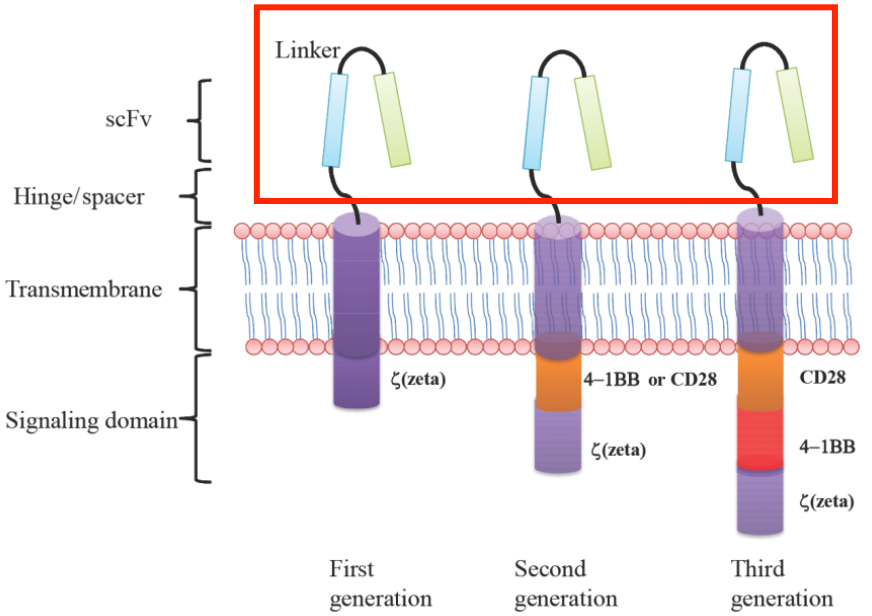
- CAR immunotherapy
- Non-human antigen recognizing domain
  - Immunogenicity upon infusion
- Our aim: reduce immunogenicity



Dai et al. 2016

Question

Which features contribute to the CAR T-cell immunogenicity?



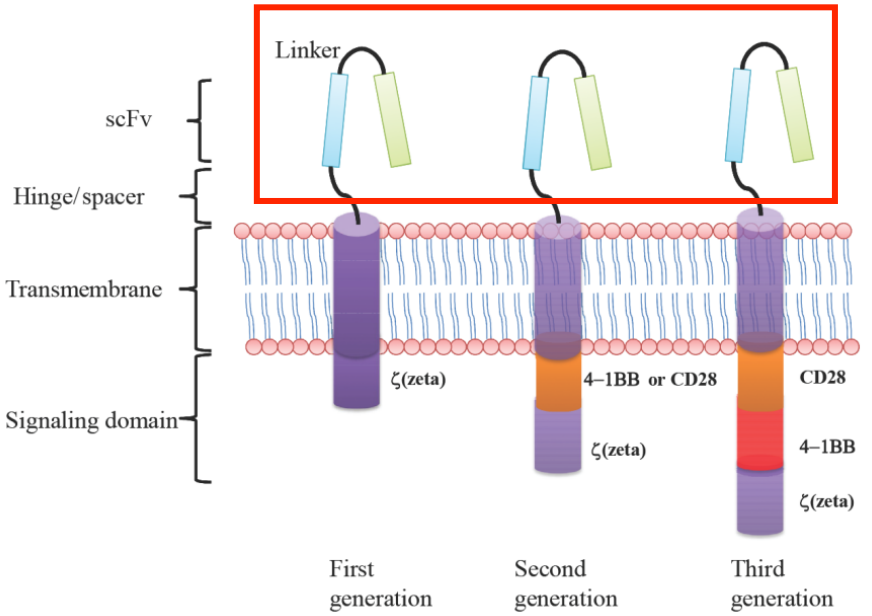
Dai et al. 2016

Question

Which features contribute to the CAR T-cell immunogenicity?

Can you propose

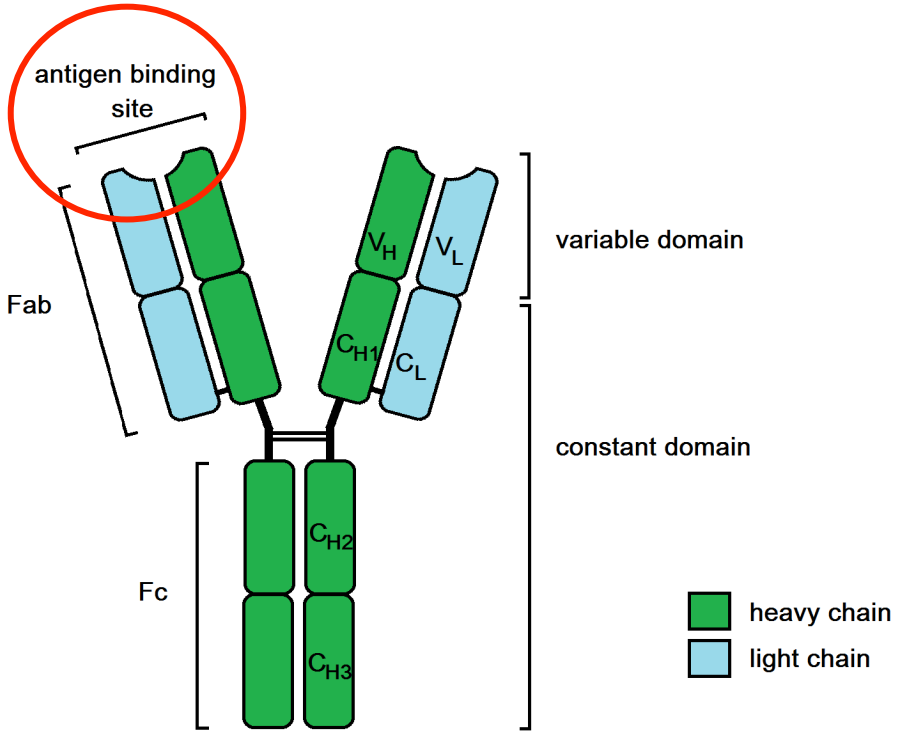
a de-immunisation strategy?



Dai et al. 2016

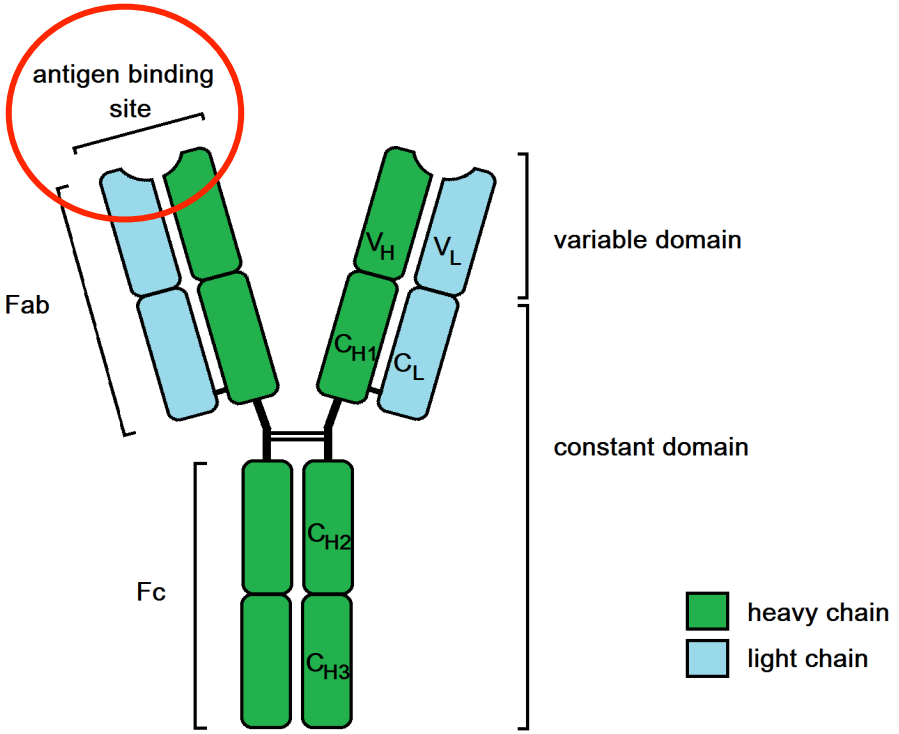
Humanization

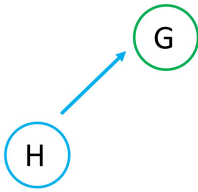
- CDR grafting
  - Antigen binding specificity
- Backmutations
  - Binding profile
- TabHu



Immunogenicity

- Test immunogenicity of humanized structure
  - “New epitopes”
- T-cell epitope prediction
  - Class I: NetMHCpan
  - Class II: NetMHCIIpan
  - Strong binders = actually binding

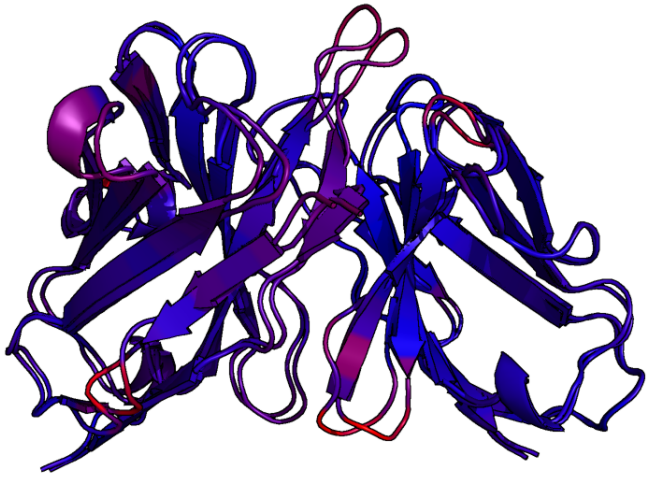




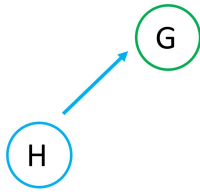
Results: Humanization

- CDR grafting
  - Germline framework
  - G1 and G2, differ in H-chain
- G1 vs. G2
- Interface type (L39-44)
  - Murine = type B
  - Framework = type A

G1 and murine



G2 and murine

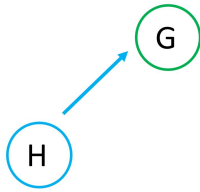


Results: Humanization

- 37 new epitopes
- 2 class 2 epitopes for G1

	Analysis of LYRA modelled CDR grafted CARs								
	FoldX minimzation (kcal/mol)	VDW strain before	VDW strain after	RMSD before	RMDS after	Class 1 SB epitopes		Class 2 SB epitopes	
G1	247.95 → 57.48	203.73	141.36	1.833	1.747	27 (H)	12 (L)	19 (H)	36 (L)
G2	147.97 → 12.96	208.33	153.36	1.795	1.669	25 (H)		9 (H)	

- Selecting G2



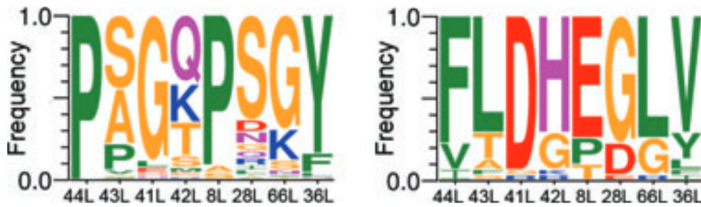
Results: Humanization

- Backmutations
  - More similar binding profile
  - Interface type A → B

Backmutations on G2

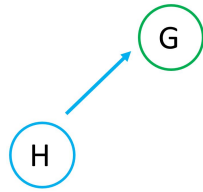
Round	Chain	Posistion	Residue H/L	Residue M	Score	Distance (Tabhu)
1	H	94	R	K	0.295	456.293
	L	80	P	Q	0.588	
2	H	42	G	R	0.207	353.022
	L	-	-	-	-	
3	H	-	-	-	-	338.849
	L	44	P	V	0.056	
	L	41	G	D	0.000	

	Interface type					
	39	40	41	42	43	44
L-murine	K	P	D	G	T	V
L-hum-G2	K	P	G	K	A	P



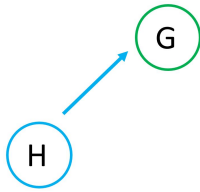
Chailyan et al. 2011

T cell epitopes



Human

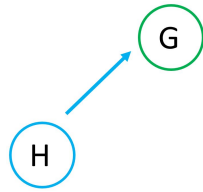
Mouse



T cell epitopes

SPAGHETTI

T cell epitopes



SPAGHETTI
STAGHETTI

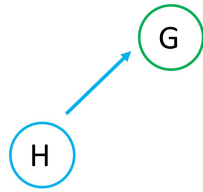
:'(

T cell epitopes

SPAGHETTI

KARTOFLER

:(

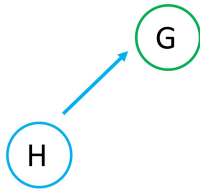


T cell epitopes

SPAGHETTI

SPAGLETTI

:|



Results: Immunogenicity

- Backmutations
- T-cell epitopes

	Class I strong binder epitopes for hum-G2				
	Allele	Core	Location	Frame-work epitope	G2 epitope
H	HLA-B*07:02	WIRPPRKAL	FR2	0.2	WIRPPGKAL$
L	HLA-B*15:01	SQQEDIATY	FR3	SLQPEIATY	SLQPEIATY*
L	HLA-B*15:01	LQQEDIATY	FR3	SLQPEIATY	SLQPEIATY*
L	HLA-B*15:01	LQQEDIATY	FR3	LQPEDIATY	LQPEDIATY*
	Class II strong binder epitopes for hum-G2				
L	DRB1_1301	LIYHTSRLH	FR2-CDR2	Not predicted	no
L	DQA10201-DQB10202	FTISSLQQE	FR3	Not predicted	FTISSLQPE*
L	DQA10401-DQB10402	FTISSLQQE	FR3	Not predicted	FTISSLQPE*
L	DQA10501-DQB10201	FTISSLQQE	FR3	Not predicted	FTISSLQPE*

$ = H42, * = L80

- Final backmutations: H94, H42, L44, and L41
- Reduce immunogenicity

Conclusion

- Balance between affinity, human-ness, and T-cell epitopes on the population