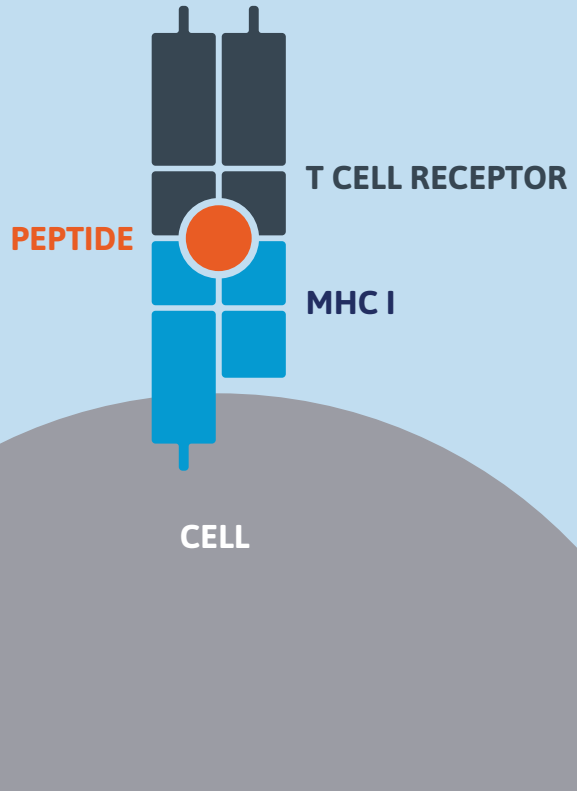
IMMUNITRACK

TCR – off-target assessment

Does your TCR bind other peptide/MHC targets?

TCR based therapies targeting peptide/MHC complexes are attractive because they can be used to target intracellular antigens.



With any T Cell Receptor (TCR) or "TCR like" based therapy there is the possibility of unwanted cross reactivity to peptide/MHC complexes that may be different in linear sequence but share molecular structure.

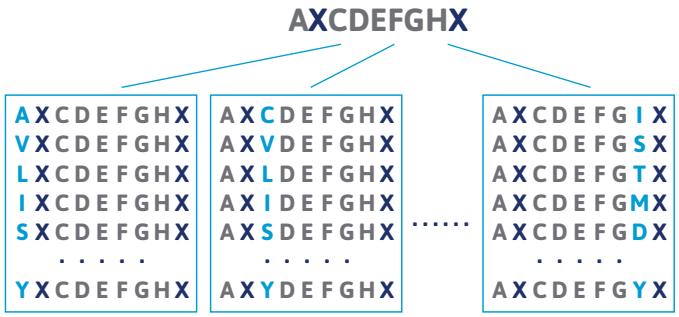
As a way of example a soluble affinity matured TCR targeting a MAGE3 derived peptide presented by A*0101 was shown to cross react with a titin derived peptide resulting in cardiac arrest and death in two patients during clinical trial.

Investigating potential off-target effects is an important pre-clinical activity and Immunitrack has a solution to it.

TCR – off-target assessment

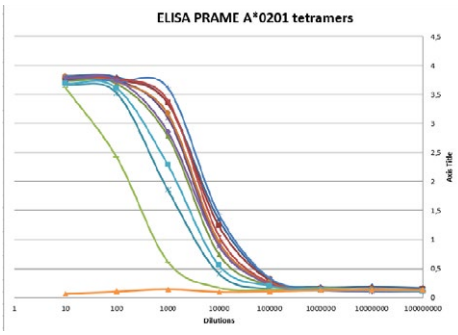
A Positional scanning library (PSL) from target peptide

A positional scanning library (PSL) is created from target peptide. Anchor positions in position 2 and 9 can be kept fixed.



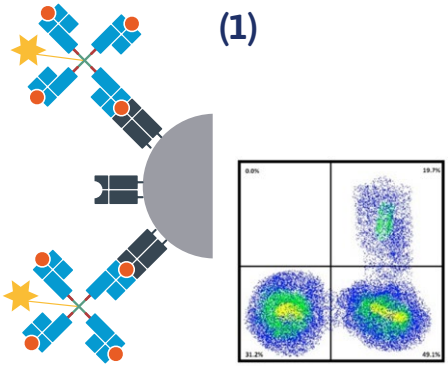
B Microscale refolding of MHC complexes from PSL peptide library

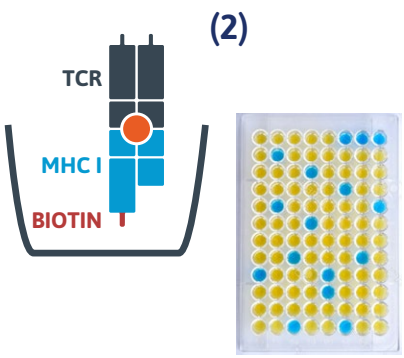
7x20 = 140 PSL peptides are refolded with relevant MHC and quantitated by ELISA. This will yield PSL/MHC complexes in sufficient amounts for in vitro testing.



C Testing TCR binding to PSL peptide MHC complexes (PSL/MHC)

Interaction between TCR and PSL/MHC complexes can be investigated either by
(1) FLOW CYTOMETRY or
(2) ELISA





D TCR PSL/MHC interaction matrix

Data from Flow or ELISA is used to construct a substitution matrix. Matrix is used to create a bioinformatic filter.

	1	2	3	4	5	6	7	8	9
A	3	X	0	0	0	0	3	0	X
R	0	X	3	3	0	0	0	3	X
N	0	X	0	0	0	0	1	0	X
D	0	X	0	3	3	0	0	0	X
C	2	X	3	0	0	3	1	0	X
E	0	X	0	0	3	0	0	0	X
Q	0	X	0	0	0	0	0	0	X
G	3	X	0	0	0	0	3	0	X
H	1	X	0	3	0	0	0	3	X
I	3	X	0	0	0	0	3	0	X
L	3	X	0	0	0	0	3	0	X
K	0	X	3	3	0	0	0	3	X
M	0	X	0	0	0	0	0	0	X
F	0	X	0	0	0	3	1	0	X
P	0	X	1	0	1	0	0	0	X
S	2	X	0	0	0	0	0	3	X
T	0	X	1	0	0	0	0	0	X
W	0	X	0	0	1	3	0	0	X
V	0	X	0	0	0	0	3	0	X
Y	0	X	1	0	0	3	0	0	X

E Finding potential cross reacting peptides in the human genome

Bioinformatic filter based on interaction matrix is used to search through the human genome for potential cross reacting peptides. Potential hits are refolded with MHC and tested in vitro as in **C**.

	Priority
sortilin-related receptor preproprotein [Homo sapiens]	55%
gp250 precursor [Homo sapiens]	55%
unnamed protein product [Homo sapiens]	55%
unnamed protein product [Homo sapiens]	55%
unnamed protein product [Homo sapiens]	55%
180 kDa transmembrane PLA2 receptor precursor [Homo sapiens]	77%
secretory phospholipase A2 receptor isoform 1 precursor [Homo sapiens]	77%
secretory phospholipase A2 receptor isoform 3 precursor [Homo sapiens]	77%
secretory phospholipase A2 receptor isoform X2 [Homo sapiens]	77%
putative soluble PLA2 receptor precursor [Homo sapiens]	77%
secretory phospholipase A2 receptor isoform 2 precursor [Homo sapiens]	77%
unknown [Homo sapiens]	77%
secretory phospholipase A2 receptor isoform X3 [Homo sapiens]	77%
secretory phospholipase A2 receptor isoform X4 [Homo sapiens]	77%
tetratricopeptide repeat protein 12 isoform X1 [Homo sapiens]	77%
secretory phospholipase A2 receptor isoform X5 [Homo sapiens]	77%