'Goldilocks' treatment window could lead to cancer therapy without harmful side effects

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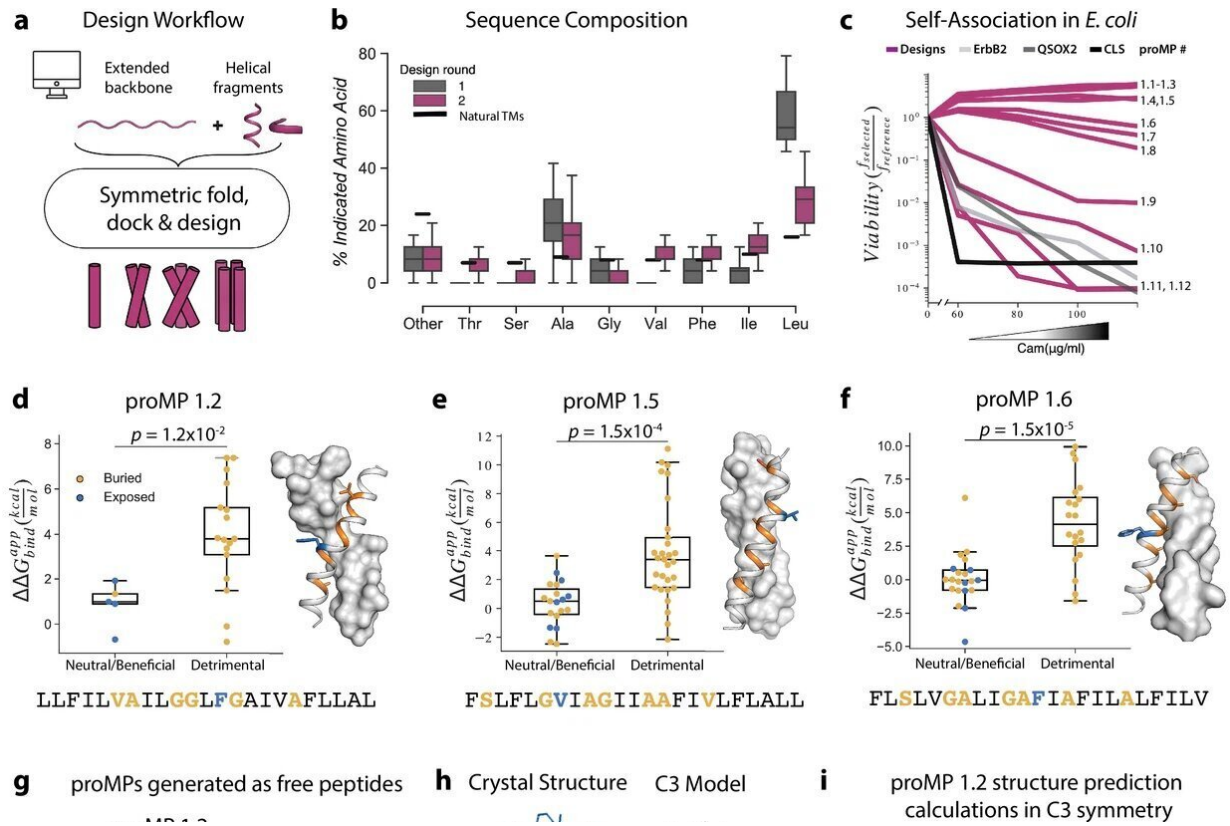


Learning the rules for programming self-associating membrane proteins (MPs). (**a**) Rosetta fold, dock, and design uses backbone fragments from natural MPs to construct symmetric, de novo architectures and an MP energy function (Weinstein et al., 2019) to optimize the amino acid sequence. (**b**) Round 1 designs were biased towards the hydrophobic amino acid Leu relative to naturally occurring transmembrane domains (TMDs). In round 2, we incorporated a sequence diversification step that conformed the amino acid propensities to those observed in natural TMDs. (**c**) The programmed membrane

proteins (proMPs) strongly self-associate in the *E. coli* inner membrane as evidenced by high viability in the deep sequencing TOXCAT-β-lactamase (dsTβL) self-association assay (Elazar et al., 2016a). The TMDs from human quiescin sulfhydryl oxidase 2 (QSOX2) and ErbB2 provide positive controls for TMD self-association, whereas the C-terminal portion of human L-selectin (CLS) provides a negative control. (**d–f**) Designed positions that are buried at the interface (orange) are more sensitive to mutation according to dsTβL analysis (Elazar et al., 2016a) (*y*-axis) than exposed positions (blue). Mutations are predicted to be detrimental or neutral/beneficial using computational mutation scanning of the model structures (Materials and methods). Changes in self-association energies upon mutation are computed according to Equation 9. (**g**) proMPs produced as free peptides form SDS-stable homo-oligomers. SDS-PAGE samples containing approximately 15, 45, and 135 μg of peptide were heated to 95°C for 1 min and run under reducing conditions. * indicates the position of a minor contaminant from the fusion protein used to generate proMP peptides (Materials and methods). Molecular weight below each gel is for a monomer of the corresponding peptide sequence with additional N-terminal EPE and C-terminal RRLC flanking sequences (Materials and methods). (**h, i**) The 2.55 Å resolution structure (blue ribbon) determined from crystals grown in monoolein lipid cubic phase (LCP) shows that proMP 1.2, designed to form a dimer, associates to form a trimer in a lipid bilayer environment. (**i**) Forward-folding ab initio prediction of proMP 1.2 in trimeric (C3) symmetry results in a model structure (**h**, gray ribbon) that is very close to the experimentally determined one. Credit: *eLife* (2022). DOI: 10.7554/eLife.75660

Researchers have developed a way to potentially reduce the toxic side-effects of a type of immunotherapy, in findings that could overcome the pioneering treatment's biggest limitation.

CAR T cell therapy is a new form of immunotherapy that enhances a patient's killer immune cells to attack and eliminate cancer.

It can be up to 90% effective in certain blood cancers and can even

deliver long-term remissions and cures in some patients. But a significant limitation is the treatment's harmful side-effects, with about 50% of patients experiencing dangerous complications.

The new study, led jointly by WEHI researchers in collaboration with Israel's Weizmann Institute of Science, has designed a way to identify a "goldilocks" window that strikes a balance of safety and efficacy.

The team's approach finetunes the cells used in the immunotherapy so that their activity is strong enough to eliminate the cancer but not so strong that they generate toxic side-effects.

The findings, led by WEHI's Associate Professor Matthew Call and Associate Professor Melissa Call, are published in *eLife*.

Crucial redesign

CAR T cell therapies involve collecting T cells from a cancer patient and supercharging the cells by individually re-engineering them in the laboratory. These enhanced cells are then put back into patients.

The T cells are engineered to produce proteins on their surface called chimeric antigen receptors (CARs), which act as artificial sensors that enable T cells to recognize and bind to specific proteins on the surface of cancer cells more efficiently.

Associate Professor Matthew Call said this synthetic sensor is what gives T cells the enhanced ability to attack and eliminate threats, like cancer cells.

"While putting these supercharged T cells into a patient with a high tumor burden can swiftly eradicate cancer cells, it also creates the perfect storm for an ongoing toxic response that can be harmful,"

Associate Professor Call said.

There is currently no way of reliably predicting how strong CAR T cell therapy will be for a patient.

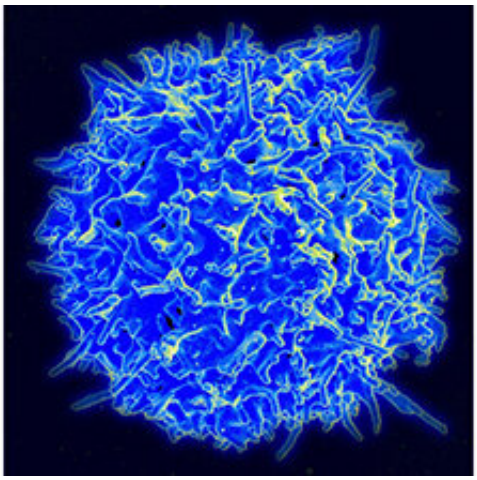
While previous studies have attempted to fine-tune T cells by targeting the end sections of the sensor, which either bind to the cancer cell or instruct the T cells to kill, the new research is the first to look at completely redesigning the middle part.

Researchers leveraged the computational expertise of the Weizmann Institute of Science to stitch together pieces of natural immune sensors with custom-designed synthetic elements, to generate new circuits that could be used to tune and assess variations of potency.

"Focusing on the connector fragment in the middle allows us to generate different versions of CARs that we know are stronger or weaker, enabling us to customize them to a patient's potency requirements," Associate Professor Call said.

"Being able to predictably tune this T cell activity significantly broadens our research, contrary to previous studies, because we are targeting something that exists in every immunotherapy scenario.

"For the first time, we can establish rules that will be applicable to any cancer where CAR T cell immunotherapy is being used."



A healthy human T cell. Re-eingineered T cells are put back into a patient during CAR T cell therapy. Credit: National Cancer Institute (NCI)

Enhanced treatment

Associate Professor Melissa Call said the ability to fine-tune T cells would dramatically reduce the number of patients experiencing severe side-effects from the treatment, which can include fever, high blood pressure and respiratory distress.

"CAR T cell therapy has proven effective in eradicating very advanced leukemias and lymphomas, while also keeping the cancer at bay for many years—even after a patient has stopped taking cancer medication," Associate Professor Call said.

"The therapy has incredible potential for cancer patients, but is currently used as a last resort due to these potentially severe side-effects.

"Our tools could lead to a fundamental rethink of the way CAR T cell therapy is offered by reducing a patient's exposure risk to harmful side-effects. This would allow patients with a broad range of cancers to be

given CAR T cell therapy far earlier in the treatment process."

There are currently over 600 clinical trials of CAR T cell immunotherapy, with the treatment already being used for several blood cancers.

Researchers hope their new tool could be used to triage immunotherapy patients based on the level of potency they require in early phases of their treatment and bring the field closer to striking that "goldilocks" treatment window for many different cancers.

The next research phase, supported by the NHMRC, the Leukemia Foundation, Cancer Australia and Hearts and Minds Investments Ltd and TDM Foundation will focus on progressing these findings into a clinical setting to see CAR T cell therapy used as a safer, first-line treatment.

More information: Assaf Elazar et al, De novo-designed transmembrane domains tune engineered receptor functions, *eLife* (2022). DOI: 10.7554/eLife.75660

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