Making any gene targetable

The OMNI™ panel of novel engineered nucleases **answers key needs** in genome editing

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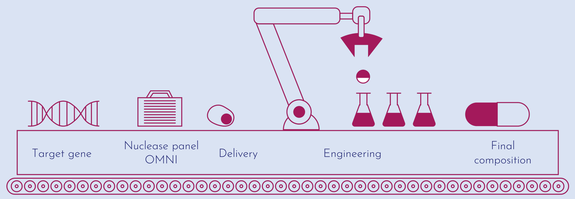
emendobio

NEXT GENERATION CRISPR
MAKING ANY GENE **TARGETABLE**

The OMNI™ nuclease panel- making any gene targetable

Successful gene editing requires developing a carefully optimized composition per target gene and target organ. Access to a variety of nucleases and guides, characterized by high activity and specificity and applicable PAM, coupled with the right delivery tool is key. Different editing objectives require specific nuclease characteristics, such as nuclease size or PAM usage, while exhibiting superb specificity. Emendo Biotherapeutics has developed a dual platform technology combining a discovery pipeline and cutting-edge protein-engineering capabilities, supported by extensive computational and machine learning tools.

Using this powerful platform, we have generated a panel of novel nucleases OMNI™ that are used in our own clinical programs, unlocking the full potential of genome editing. These Type II nucleases are diverse in size and compatible with all delivery modalities; PAM usage diversity allows for 86% genome coverage; and highly specific engineered variants enable allele specific editing. Together all of the above makes any gene targetable.



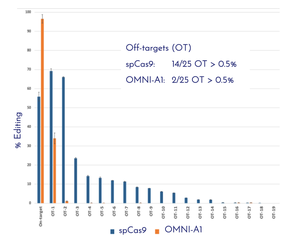
Safety

Super-specific and highly active OMNI™ nucleases

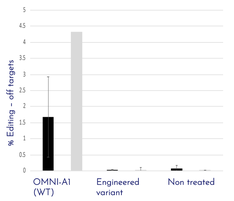
- No off-target effects
- No translocations
- Allele specific editing

OMNI-A1 was identified using Emendo Biotherapeutics' nuclease discovery platform, exhibiting high editing activity as well as low off target effects. Due to the key safety aspect of off-target effects, OMNI-A1 was optimized using the engineering platform to eliminate off-target editing. CIRCLEseq analysis found OMNI-A1-V10, an engineered variant of OMNI-A1, to have significantly less off-target effects than the wild-type nuclease or spCas9. Importantly, no Off-Target Mediated Translocations (OMTs) were detected using unbiased CASTseq analysis of OMNI-A1-V10 in target cells.

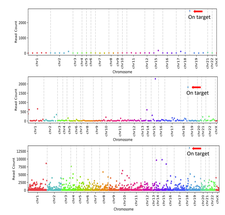
OMNI-A1 – highly active and specific



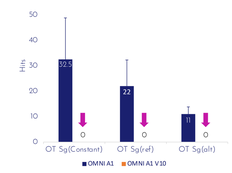
OMNI-A1-V10 - engineered to eliminate off target effects



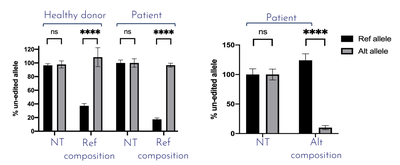
CIRCLEseq analysis shows **OMNI-A1-V10** to have significantly less off-target effects than the pre-engineered nuclease or spCas9



No Off-Target Mediated Translocations (OMTs) detected following **OMNI-A1-V10** editing



OMNI-A1-V10 allows for fully discriminatory allele specific editing*



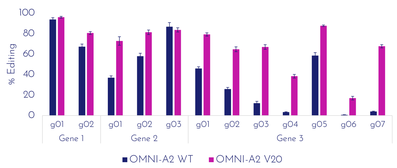
* Mutant allele knockout with novel CRISPR nuclease promotes myelopoiesis in ELANE neutropenia. Sabo *et al.* Mol Ther Methods Clin Dev. 2022.

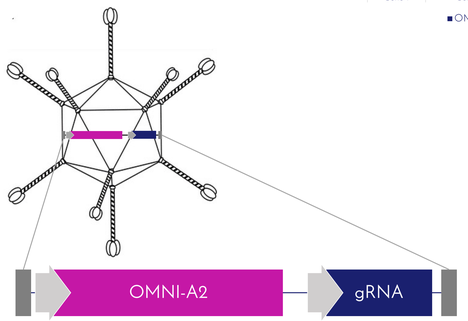
Compatible with all delivery modalities

Short AAV-delivery-compatible nucleases - **OMNI-A2** (~1,050aa)

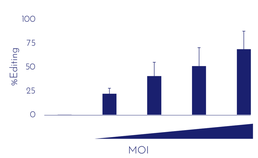
Adeno Associate Virus (AAV) based vectors are an important part in the delivery toolbox but have limited payload capacity. Using its dual discovery platform, Emendo Biotherapeutics has identified short CRISPR nucleases compatible with AAV packaging. Protein engineering was used to further improve the editing efficiency of these short nucleases, as exemplified by OMNI-A2 and its hyper active variant OMNI-A2-V20.

OMNI-A2 and engineered **OMNI-A2-V20**
Highly active, short, AAV packaging compatible novel nucleases





AAV delivered **OMNI-A2** editing



Genome accessibility

OMNI™ Nuclease panel to overcome PAM Constraints

The diversity of OMNI™ nucleases particularly PAM site diversity widens significantly genome accessibility and enables targeting of genomic sites that are not accessible using NGG nucleases. Accumulatively the OMNI™ panel covers approximately 86% of the genome, making any gene targetable.

Short nucleases

OMNI™	PAM	Length (aa)	MaxEdit
OMNI-A2	NGGNNNNN	1062	0.90
OMNI-A7	NNGNRCNN	1097	0.87
OMNI-A8	NYAGCNNN	1109	0.88
OMNI-A13	NNNNCMAN	1091	0.91
OMNI-A31	NNGRVNNN	1054	0.86
OMNI-A34	NNNNCVTA	1078	0.91

Long nucleases

OMNI™	PAM	Length (aa)	MaxEdit
OMNI-A1	NGGNNNNN	1370	0.98
OMNI-A3	NNGNRANN	1154	0.81
OMNI-A54	NRRNATNN	1107	0.85
OMNI-A4	NNRACTTN	1348	0.93
OMNI-A8	NYAGCNNN	1109	0.88
OMNI-A21	NRGGGCRN	1215	0.89

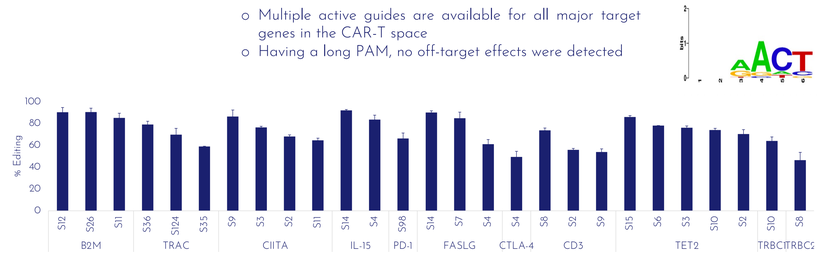
Major IP issue in the cell therapy space: heavily patented NGG guides

A little recognized intellectual property (IP) limitation is that of patented guide sequences. For many gene targets, for example CAR-T gene targets, multiple patents by various entities cover applicable NGG guide sequences, making them unavailable for companies that desire using CRISPR as their editing tool. Using OMNI non-NGG nucleases and guides overcomes guide IP barriers.

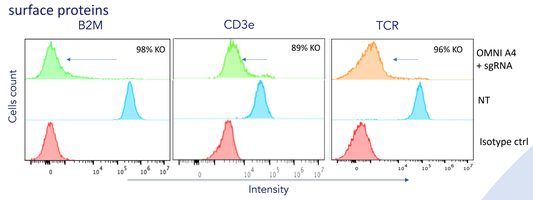
Gene name	# of patent families directed to guide sequences	# of NGG guides left after patent screen	# of guides for non-NGG nucleases selected for Emendo Biotherapeutics' screening
TRAC	56	0/20	15
TRBC1	23	1/20	3
TRBC2	23	1/21	5
CD3e	4	10/79	18
B2M	42	0/33	12
CIITA	9	0/294	35
PD1	51	0/196	29

OMNI-A4: non-NGG PAM nuclease compositions for major targets in the cell therapy and immuno-oncology space

- Multiple active guides are available for all major target genes in the CAR-T space
- Having a long PAM, no off-target effects were detected



Gene knockout using **OMNI-A4** results in a dramatic reduction in expression of cell surface proteins



CAR-T ready OMNI™ and guide compositions

Target gene	% Editing (by NGS)
B2M	91
CD3	70
CIITA	82
CTLA4	75
FASLG	68
HAVCR2	94
HLA-E	94
PD1	44
TET	88
TRAC	90
CISH	70

We are open to collaboration on the OMNI™ nuclease panel and editing capabilities

For further information please contact us