editSTAT3

Preclinical development of TALEN®-based genome editing
in T cells for the treatment of Hyper-IgE-Syndrome

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ESGCT Congress 2021

AD-HIES

Autosomal-Dominant Hyper IgE Syndrome

Primary immunodeficiency disorder

- ❖ dominant / haploinsufficiency
- ❖ associated with significant morbidity and mortality (life expectancy ~50 years)
- ❖ prevalence 1-9 per 100,000 worldwide
- ❖ 372 registered patient in ESID system

- ❖ <u>disease manifestations:</u>
    - ❖ chronic eczema
    - ❖ recurrent Staphylococcal infections
    - ❖ increased serum IgE and eosinophilia (IgE >2000 IU/ml; normal: 5-350 IU/ml)
    - ❖ distinctive coarse facial appearance, abnormal dentition, hyperextensibility of the joints, bone fractures

- ❖ <u>treatment options:</u>
    - ❖ antibiotic prophylaxis
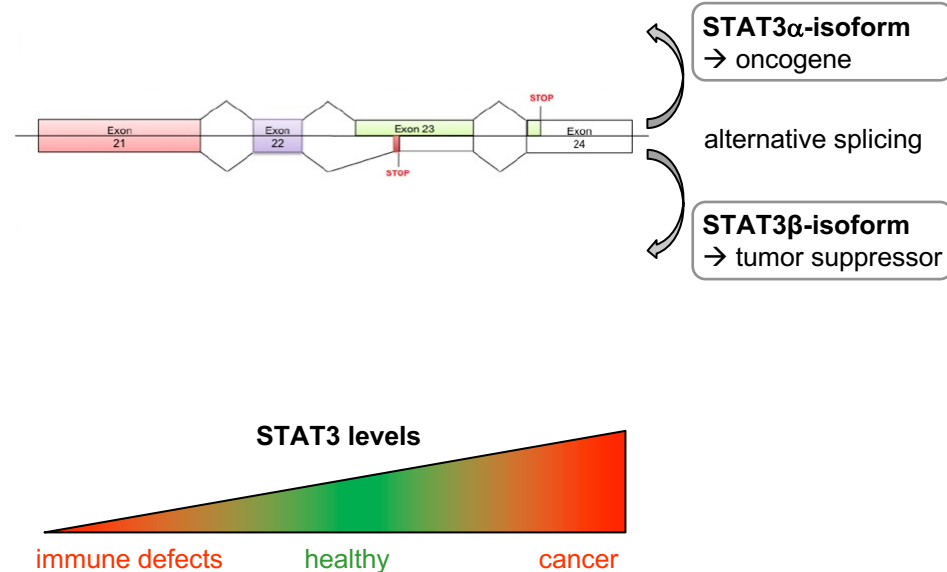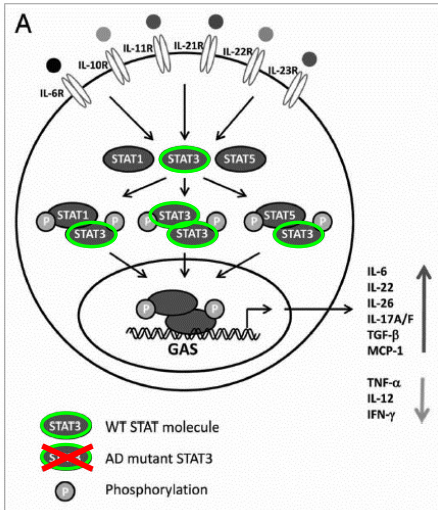   - ❖ antifungal drugs

  - ➔ no curative treatment available
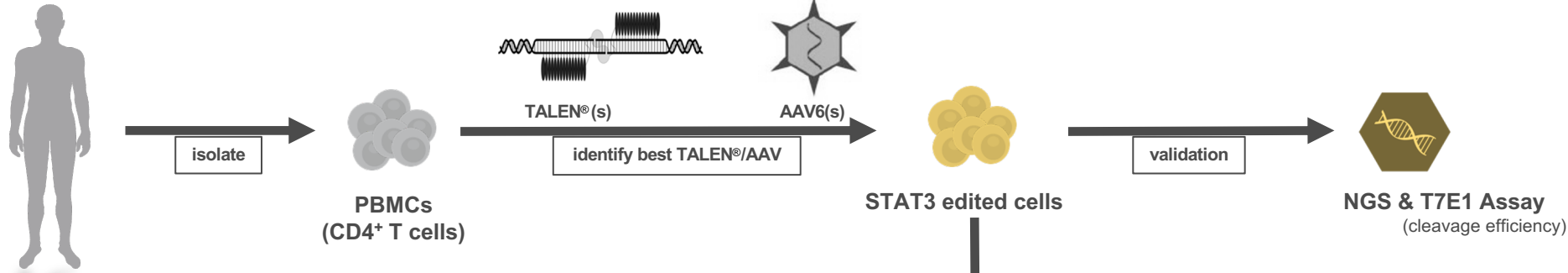 - ➔ allogenic hematopoietic stem cell transplantation (HSCT) not recommended

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STAT3 Signaling

STAT3 mutations associated with dysregulation of many cytokines

STAT3α-isoform
→ oncogene

alternative splicing

STAT3β-isoform
→ tumor suppressor

STAT3 levels

immune defects   healthy   cancer

➔ prime example for genetic disorders caused by mutations in tightly regulated genes

➔ challenge for gene therapy approaches

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Research plan

isolate

PBMCs
(CD4+ T cells)

TALEN®(s)

identify best TALEN®/AAV

AAV6(s)

STAT3 edited cells

validation

NGS & T7E1 Assay
(cleavage efficiency)

healthy donor/
AD-HIES patient

transplantation

Aim:
pre-clinical development of
TALEN®-based genome editing in
long-lived T cells for the treatment
of **Hyper-IgE-Syndrome**
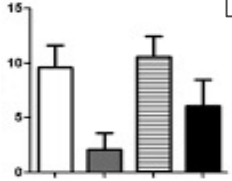
cellular   molecular

assessment of functional rescue

ddPCR
(integration)

RT-ddPCR
(expression)

WB
(expression)
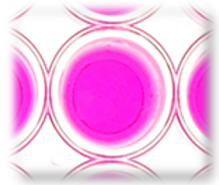
Th17
differentiation
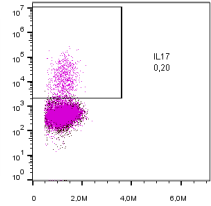
(intra)cellular
staining

IL-17
CBA Assay

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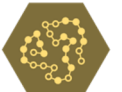
# STAT3 mutations

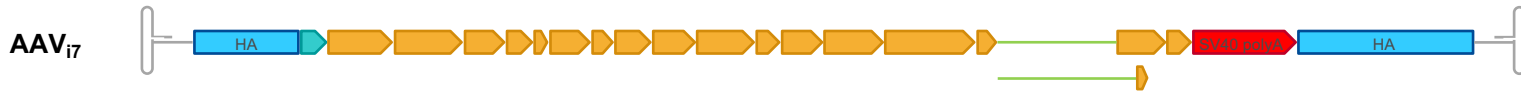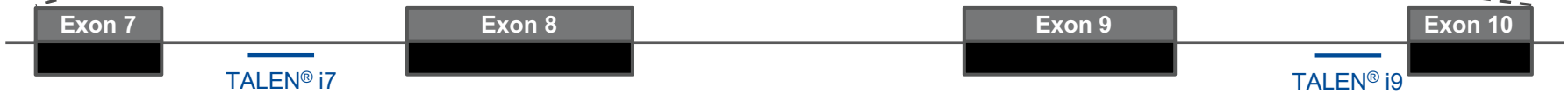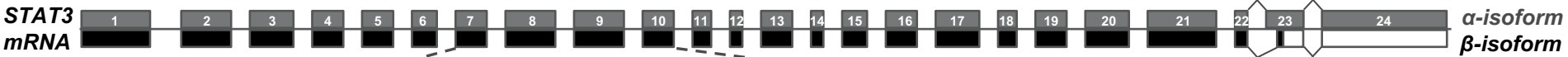## position of the mutations leading to HIES
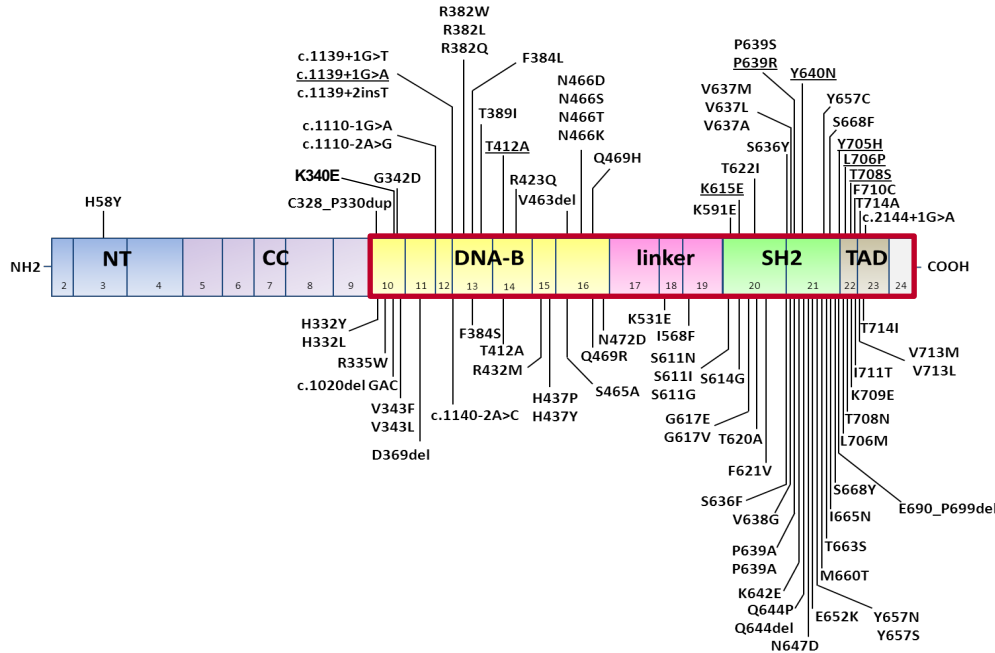
NT: N-terminal domain
CC : coiled-coiled domain
DNA-B: DNA-binding domain
linker: linker domain
SH2: Src homology 2 domain
TAD: transactivation domain

➔ ~60 different mutations with hot spots starting from exon 10

➔ **strategy:** targeted integration **before exon 10**
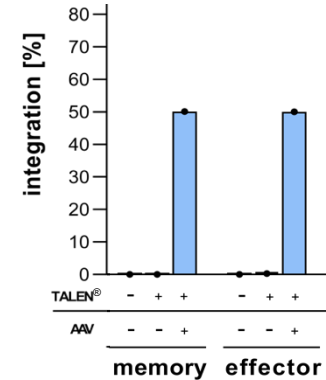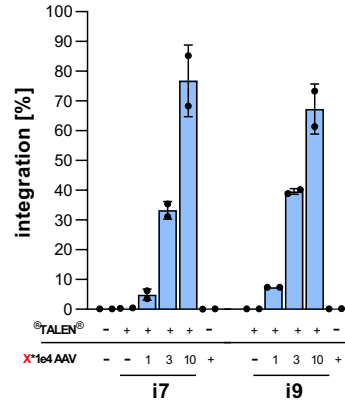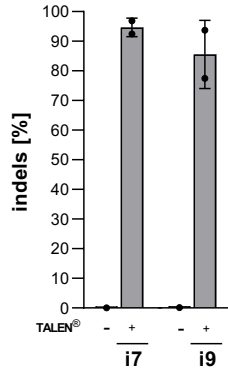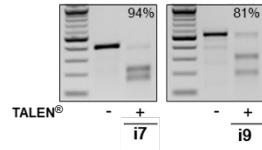
STAT3 gene editing strategy

TALEN® & SMART donor design
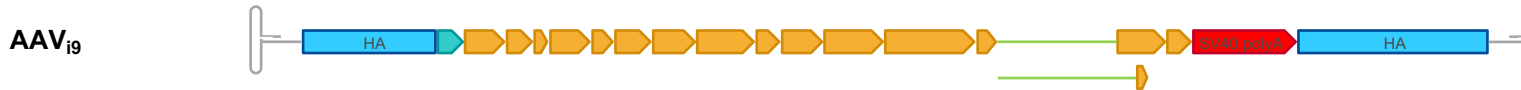
Characterization of TALENs® & SMART donors

TALEN® activity, knock-in & SMART transgene expression
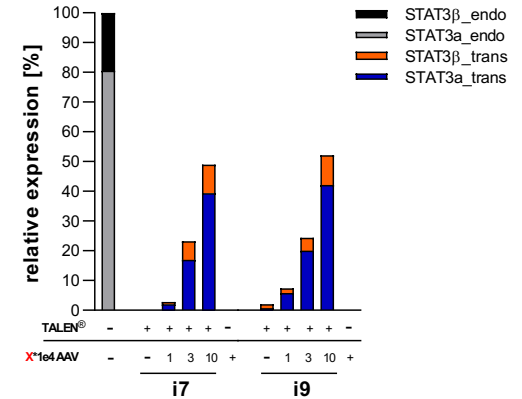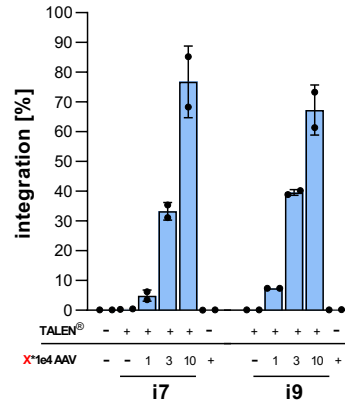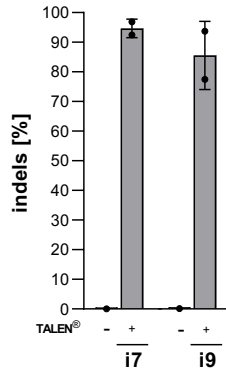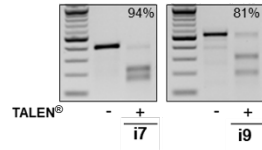

➜ ~high activity (80-90%) with both TALEN®

➜ KI in ~70% of T cells

➜ comparable integration in memory and effector T cells

Characterization of TALENs® & SMART donors

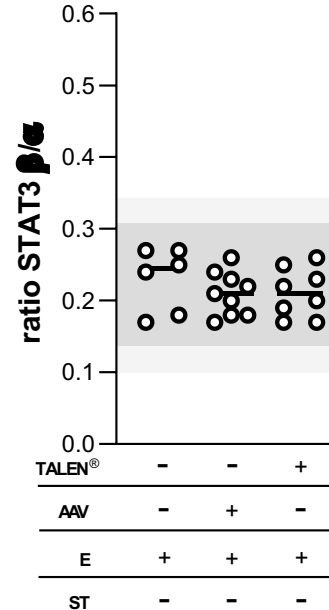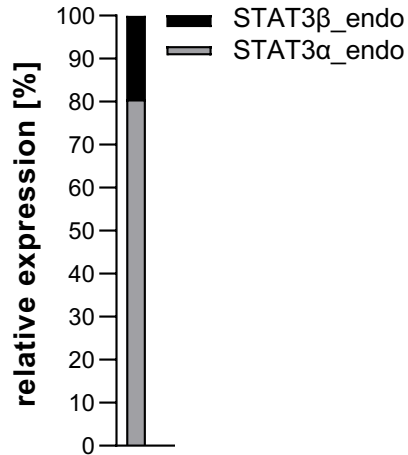## TALEN® activity, knock-in & SMART transgene expression

➔ ~high activity (80-90%) with both TALEN®

➔ KI in ~70% of T cells

➔ STAT3 transgene expression levels reached 50% of total STAT3 mRNA

Characterization of TALENs® & SMART donors

SMART transgene isoform balance

→ STAT3 isoform ratio crucial

→ both approaches lead to balanced STAT3 transgene expression
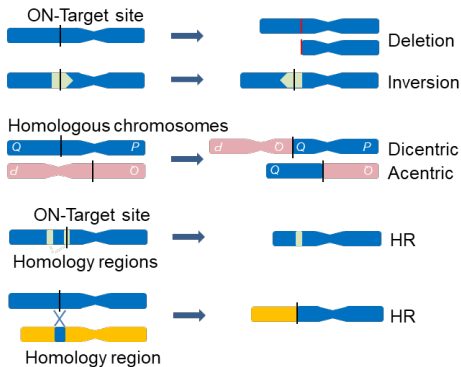
Characterization of TALENs® & SMART donors

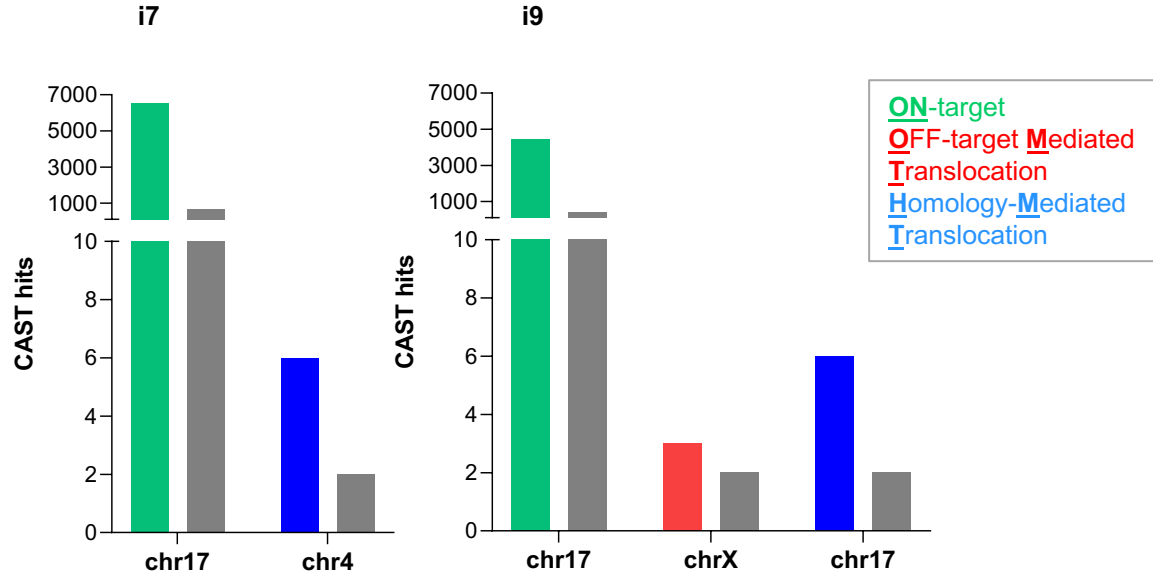## TALEN® safety assessment

CAST-Seq analysis

OFF-target mediated aberrations

ON-Target site

OFF-Target site

Translocation

ON-target mediated aberrations

ON-Target site

Deletion

Inversion

Homologous chromosomes

Q   P   d   O Q   P   Dicentric
                  Q   O   Acentric

ON-Target site

HR

Homology regions

HR

Homology region

i7

i9

ON-target
OFF-target **M**ediated **T**ranslocation
Homology-**M**ediated **T**ranslocation
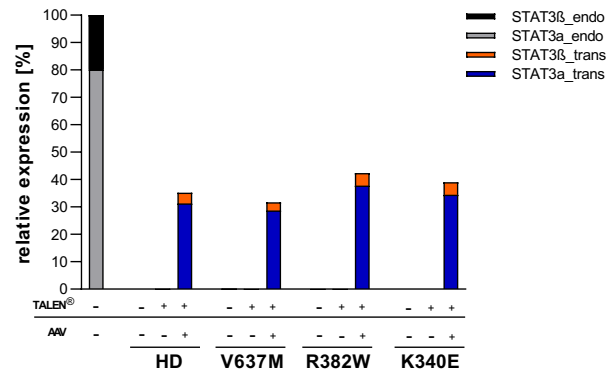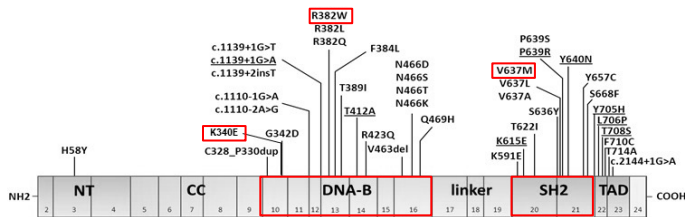
➔ detection of **OFF-** and **ON-target** mediated aberrations

➔ high TALEN® specificity (OT ≤0.02%, no oncogene)
➔ continue with **i7** approach

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Correction of HIES patient-derived cells

Freiburg HIES cohort (n=3)
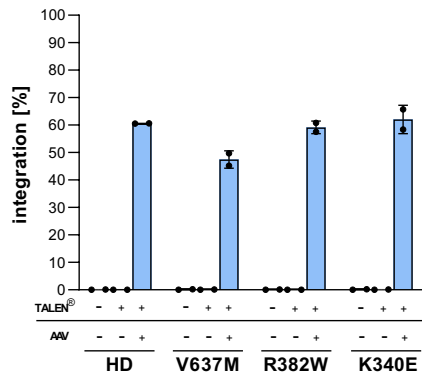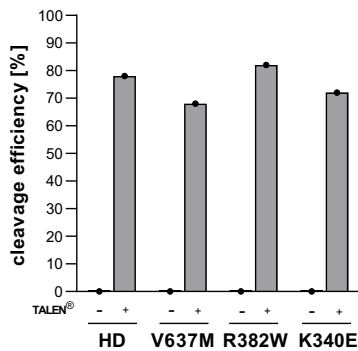
→ ~70% cleavage efficiency

→ ~60% integration

→ ~35-45% transgene expression

Summary and Outlook

proof of concept in HD PBMCs:

✓ ❖ successful establishment of read-out systems for characterization, integration and transgene expression

✓ ❖ successful validation of the best **TALEN®/AAV6 combination**

(high specificity, ~80% cleavage efficiency, ~50% transgene expression)

✓ ❖ **isoform splicing** from the transgene and **isoform-ratio** is maintained

✓ ❖ targeted integration shown for both effector and **long-lived memory T cells**

targeted integration in HIES patient-derived PBMCs:

✓ ❖ successful characterization of PBMCs from 3 HIES patients

✓ ❖ high cleavage efficiency (~70%)

✓ ❖ successful targeted integration (~60%) and transgene expression (~35-45%)

next step:

❖ functional validation of correction in HIES patient PBMCs

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Acknowledgments