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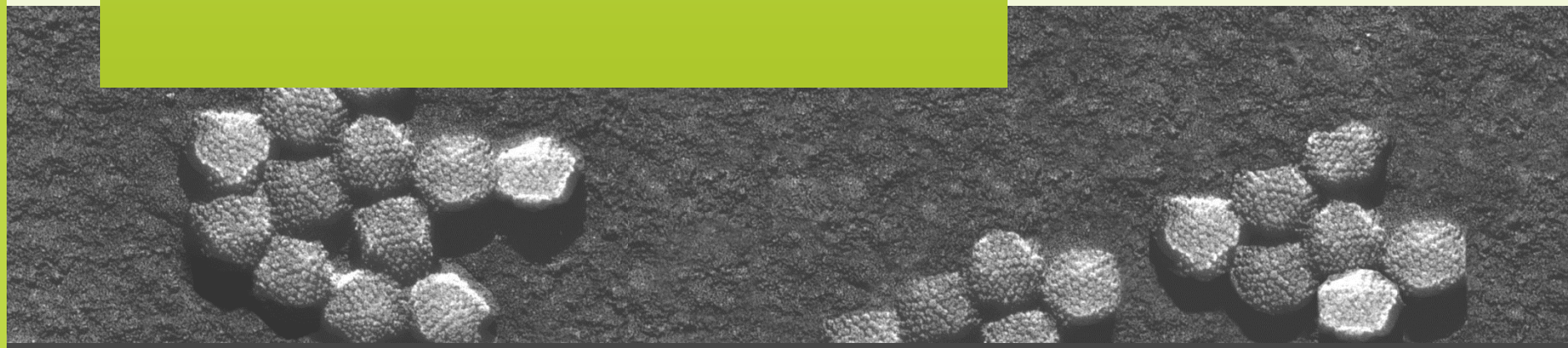
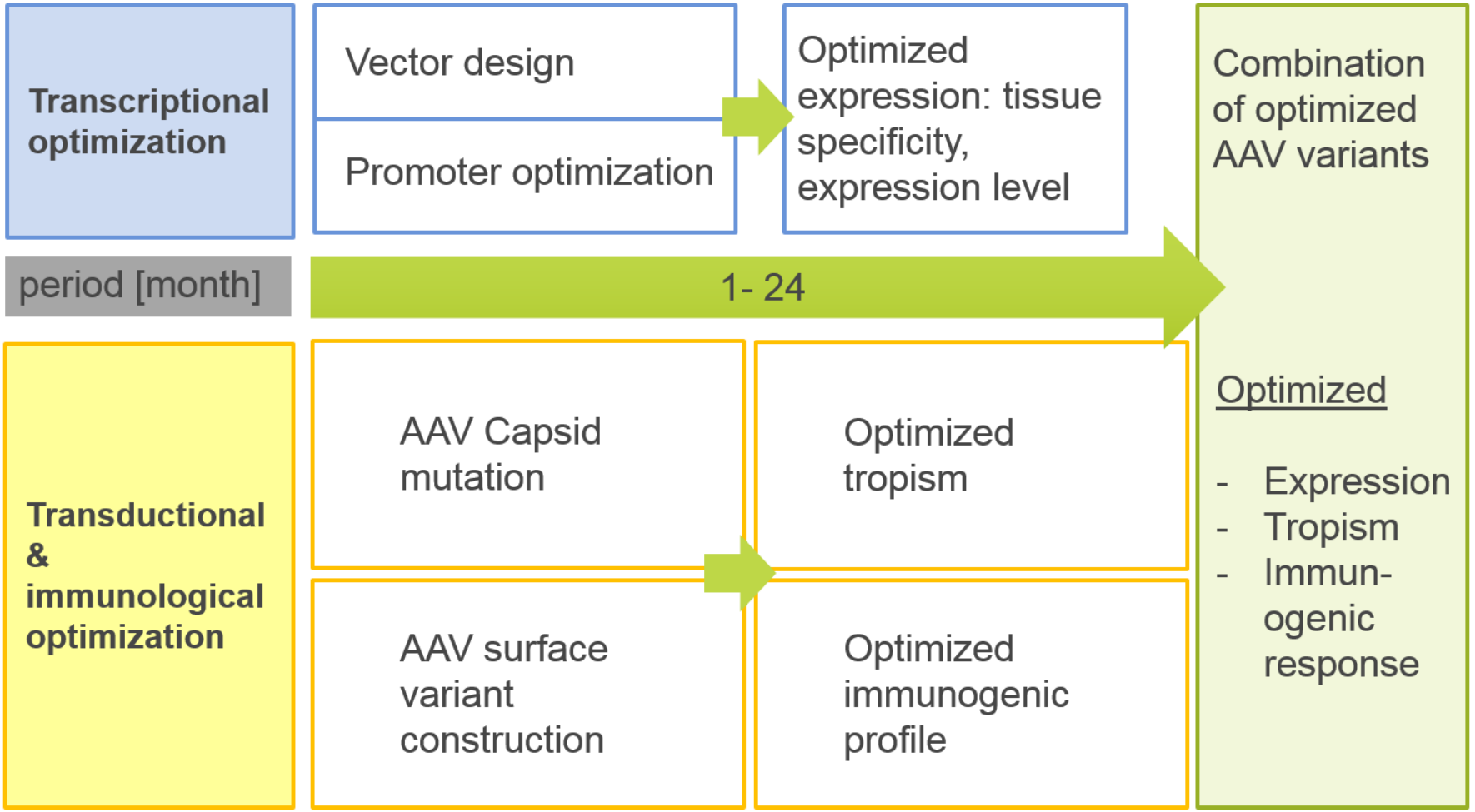
Optimization of AAV tissue specificity & immunogenic profile

- project overview



OPTIMIZATION OF AAV TISSUE SPECIFICITY & IMMUNOGENIC PROFILE – TRANSDUCTIONAL & TRANSCRIPTIONAL APPROACH



TRANSDUCTIONAL & TRANSCRIPTIONAL OPTIMIZATION

I. GENERATION OF ENGINEERED AAV VARIANTS WITH INCREASED TRANSDUCTION EFFICIENCY

1. AAVx random peptide insertion / shuffled library generation
2. Promoter structure and enhancer optimization
3. Selection of capsid variant AAVs via *in vitro* screening approaches
4. *Optional:* NGS-guided selection of capsid variant AAVs *in vivo*
5. Validation of capsid variant AAVs *in vitro & in vivo*
6. High titer production of capsid variant AAVs for validation (benchmarking against the WT AAV)

I. GENERATION OF ENGINEERED AAV VARIANTS WITH INCREASED TRANSDUCTION EFFICIENCY

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Promoter structure and enhancer optimization	NGS guided AAVx selection *in vivo*	AAV capsid mutant validation *in vitro* & *in vivo*	AAV capsid mutant validation: high titer production & benchmarking against WT AAV
AAVx library generation (5X10E6)	AAVx library selection *in vitro*		

I. Generation of engineered AAV variants with increased transduction efficiency

1-24 month

II. Generation of engineered AAV mutants with optimized immunogenic profile

TRANSDUCTIONAL OPTIMIZATION

II. ENGINEERING OF AAV CAPSID MUTANTS TO ESCAPE RECOGNITION BY NEUTRALIZING ANTIBODIES (NABS) WHILE PRESERVING THEIR TROPISM

1. Mutation of variable AAV surface regions (single mutations) + identification & mutation of AAV epitopes recognized by neutralizing antibodies
2. *In vitro* transduction verification of mutants in the presence of neutralizing antibodies, pooled IVIG, Nab-containing serum
3. *In vivo* verification of best performer mutants in the presence of Nab-containing serum
4. Combination of positively evaluated mutants: novel super-mutants with improved immune-escaping profiles
5. Validation of combined immune evasion capsid mutants for packaging efficiency and transduction and immune evasion *in vitro*
6. Validation of combined immune evasion capsid mutants *in vivo* : impact on transduction efficiency and immunogenicity

II. ENGINEERING OF AAV CAPSID MUTANTS TO ESCAPE RECOGNITION BY NEUTRALIZING ANTIBODIES (NABS) WHILE PRESERVING THEIR TROPISM

I. Generation of engineered AAV variants with increased transduction efficiency

1-24 month

II. Generation of engineered AAV mutants with optimized immunogenic profile

AAV capsid mutant construction & production

AAV capsid mutant validation in the presence of Nab containing serum: *in vitro* & *in vivo*

AAV super-mutant generation

AAV super mutant validation: production & transduction efficiency, immune evasion *in vitro*, immunogenic *in vivo* profile

III. COMBINATION OF TRANSCRIPTIONALLY, TRANSDUCTIONALLY AND IMMUNOLOGICALLY OPTIMIZED AAV MUTANTS

1. Combined vectors based on mutants from modules I and II
2. Large-scale AAV productions from variants
3. *In vitro* validation
4. *In vivo* validation

5. Deliver the best performers to client for evaluation

PROJECT STRUCTURE

SIRION BIOTECH

UniversitätsKlinikum Heidelberg

<u>Strategic Partnership SIRION / UKHD:</u>
Project management by SIRION
Scientific execution by SIRION plus UKHD
Regular working meetings
Regular & written reports

COLLABORATION AGREEMENT
CLIENT granted access to background IP and license option to improved AAVx vectors

COMMERCIAL LICENSE AGREEMENT
CLIENT granted commercialization & further research / development rights
IP rights

BACKGROUND / FOREGROUND IP

AAV vector technology background

- AAV 2-plasmid system EP 0 934 423 (granted) commercial license to SIRION
- Insertion sites for peptides AAV1-12
- AAV directed evolution knowhow UKHD
- Random peptide insertion geometry knowhow UKHD
- Assays for immune-escape phenotyping

AAV vector technology expected IPR

- Transduction and immunologically enhanced AAVx vectors

Access to IP and rights

- Technology access and license option fee
- Collaboration agreement grants access to background IP and option for exclusive licensing of improved AAV vectors in the field
- Commercial licensing agreement grants rights to use and commercially exploit improved AAVx vectors in the field

Disclaimer: No claim on completeness of information provided. Information is preliminary and shall be subject to further examination by CLIENT and SIRION

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