Response Evaluation In Neurofibromatosis Schwannomatosis
INTERNATIONAL COLLABORATION

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Scientific approaches to gene directed therapies for NF2 and Schwannomatosis

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Challenges to therapeutic intervention in NF2 and SWN

- In both conditions *NF2* is lost in tumors (despite different germline mutations: *NF2, SMARCB1, LZTR1*…)
- Benign nature of tumors precludes use of strategies targeting rapidly dividing, metabolically active cells
- Reduced therapeutic window for treatment
- Exploiting unique features of $NF2^{-/-}$ tumor cells to target the broad consequences of NF2 protein loss => gene replacement therapies?
- Would NF2 re-expression, even if successfully delivered, stop or reverse tumor growth?

- **Tumor-specific therapeutic targets**
- **Strategies for delivering therapeutics**
- **Validation of NF2 gene replacement as a viable therapeutic strategy**

=> key unmet needs for NF2 tumor therapy

Current and applicable definitions of human gene therapy

FDA (Cellular & Gene Therapy Guidances, July 20, 2018)
EU commission (Directive 2001/83/EC, Part IV of Annex I)

A biological medicinal product containing recombinant nucleic acid used in or administered to a human to regulate, repair, replace, add, or delete a genetic sequence with the aim to treat or cure diseases

The discipline of gene therapy includes:

(1) in vivo vector-mediated gene therapy

(2) ex vivo cell transduction gene therapy

(3) genome editing

Schwannomas and Meningiomas are appealing targets for gene therapy:

1) Slow growing

2) Few (if any) recurring genetic alterations in addition to NF2 loss

3) can be readily localized using magnetic resonance imaging for direct intratumoral vector injection.

Gene-therapy is potentially advantageous compared to resection:

1) Minimally invasive

2) Kills tumor cells without damaging tumor-associated nerve

3) May allow treatment of lesions not amenable to resection

Cancer Gene Ther. 2010 Apr;17(4):266-74. doi: 10.1038/cgt.2009.71. Epub 2009 Oct 16.

Imaging and therapy of experimental schwannomas using HSV amplicon vector-encoding apoptotic protein under Schwann cell promoter

S Prabhakar [1], G J Brenner, B Sung, S M Messerli, J Mao, M Sena-Esteves, A Stemmer-Rachamimov, B Tannous, X O Breakefield

Hum Gene Ther. 2013 Feb;24(2):152-62. doi: 10.1089/hum.2012.094. Epub 2013 Jan 30.

Regression of schwannomas induced by adeno-associated virus-mediated delivery of caspase-1

Shilpa Prabhakar [1], Mehran Taherian, Davide Gianni, Thomas J Conlon, Giulia Fulci, Jillian Brockmann, Anat Stemmer-Rachamimov, Miguel Sena-Esteves, Xandra O Breakefield, Gary J Brenner

Neuro Oncol. 2019 Jul 11;21(7):854-866. doi: 10.1093/neuonc/noz065.

Gene therapy with apoptosis-associated speck-like protein, a newly described schwannoma tumor suppressor, inhibits schwannoma growth in vivo

Sherif G Ahmed [1], Ahmed Abdelnabi [1], Casey A Maguire [2], Mohamed Doha [1], Jessica E Sagers [3 4], Rebecca M Lewis [3], Alona Muzikansky [5], Marco Giovannini [6], Anat Stemmer-Rachamimov [7], Konstantina M Stankovic [3 4], Giulia Fulci [1 8], Gary J Brenner [1]

Cancer Gene Ther. 2019 Sep;26(9-10):259-267. doi: 10.1038/s41417-018-0077-3. Epub 2019 Jan 9.

Schwannoma gene therapy by adeno-associated virus delivery of the pore-forming protein Gasdermin-D

Sherif G Ahmed [1], Ahmed Abdelanabi [1], Mohamed Doha [1], Gary J Brenner [2]

International Journal of Molecular Sciences 2022 MDPI

Article

Schwannoma Gene Therapy via Adeno-Associated Viral Vector Delivery of Apoptosis-Associated Speck-like Protein Containing CARD (ASC): Preclinical Efficacy and Safety

Sherif G. Ahmed [1], Casey A. Maguire [2], Shiliang Alice Cao [1] and Gary J. Brenner [1,*]

1) Gene therapy for schwannoma via direct intratumoral injection of apoptosis inducing genes

- Direct intra-tumoral injection of AAV1 vector into schwannomas grafted in the mouse sciatic nerve
- Schwann cell specific promoter (P0) protects neuronal tissue from potential off-target toxicity
- Significant reduction in tumor growth

2) Aberrant splicing correcting approach as a genotype-dependent therapeutic for NF2

Case Reports > Eur J Hum Genet. 2013 Jul;21(7):769-73. doi: 10.1038/ejhg.2012.261. Epub 2012 Nov 28.

In vitro antisense therapeutics for a deep intronic mutation causing Neurofibromatosis type 2

Elisabeth **Castellanos** [1], Imma Rosas, Ares Solanes, Isabel Bielsa, Conxi Lázaro, Cristina Carrato, Cristina Hostalot, Pepe Prades, Francesc Roca-Ribas, Ignacio Blanco, Eduard Serra, **NF2** Multidisciplinary Clinics HUGTiP-ICO-IMPPC

A deep intronic mutation responsible for an aberrant NF2 splicing could be corrected by the use of Phosphorodiamidate Morpholino Oligomers (PMOs) and recover Merlin function

Platform: Testing the Use of Antisense Oligonucleotides to Modulate the Effect of Splicing and Nonsense Genetic Variants Causing Neurofibromatosis Type 2

Tuesday, June 15, 11:45am – 12:00pm

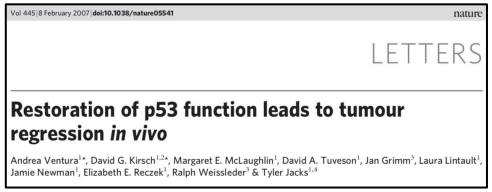
Núria Catasús, *Clinical Genomics Research Unit, Hereditary Cancer Group – PMPPC, Germans Trias i Pujol Research Institute*

PMOs may not be useful for correcting splicing when mutations are located in splicing canonical sites, since the use of these antisense molecules results in Merlin loss

=> Approach might be useful therapeutically in a subset of patients

3) Tumor suppressor gene replacement therapies

Pre-clinical studies:



Clinical trials:

P53 mutant tumors

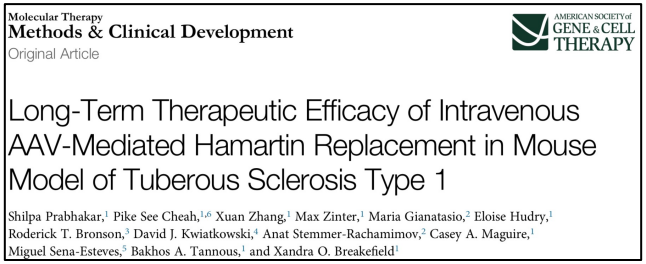
- **Gendicine™**
  - (Ad-p53) intratumoral injection
  - Enters tumor cells by receptor-mediated endocytosis => p53 overexpression
  - Shows efficacy alone and synergistically with conventional treatments, chemo- and radiotherapy.
  - p53 mutation status of the tumor cells and response to Ad-p53 treatment are not closely correlated
  - Produces fewer side effects than conventional therapy
  - Approved by China State Food & Drug Administration in 2003 for H&N squamous cell carcinoma
  - Tested in a number of clinical trials for HCC, NSCLC, malignant glioma, and epithelial ovarian carcinoma
  - **No information is available about the submission of clinical data for approval from the USFDA to date**

- **Advexin™**
  - (Ad-p53) systemic (intravenous) delivery
  - Tested in both preclinical and phase I/II clinical trials (colorectal cancer, HCC, NSCLC, prostate, breast, ovarian, bladder, glioma, and squamous cell carcinoma of H&N) and in a Li-Fraumeni patient
  - **Not FDA approval yet**

➢ **Summary Adp53 gene therapy**

- **Well tolerated, feasible**
- **Exerts promising antitumor effects in some cases,**
- **its overall clinical efficacy is not conclusive.**
- **No Adp53 therapies have been approved in the USA**

Tumor suppressor gene replacement therapies - TSC



Molecular Therapy
Methods & Clinical Development
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

Long-Term Therapeutic Efficacy of Intravenous AAV-Mediated Hamartin Replacement in Mouse Model of Tuberous Sclerosis Type 1

Shilpa Prabhakar,[1] Pike See Cheah,[1,6] Xuan Zhang,[1] Max Zinter,[1] Maria Gianatasio,[2] Eloise Hudry,[1] Roderick T. Bronson,[3] David J. Kwiatkowski,[4] Anat Stemmer-Rachamimov,[2] Casey A. Maguire,[1] Miguel Sena-Esteves,[5] Bakhos A. Tannous,[1] and Xandra O. Breakefield[1]

- TSC1 GEMM model (AAV1-Cre injected at P0)
- AVV-hamartin serotypes rh8 and 9 (cross the BBB), increased survival time by at least 3-to 13-fold.
- Increase in survival was accompanied by normalization of sizes of ventricles and neural cell bodies in the brain
- Well tolerated

Challenges of gene replacement therapy for NF2

Vector selection

- Viral (AAV, which serotype to target Schwann and arachnoid cells *in vivo*?)
- Non-viral (exploiting macropinocytosis proficiency of NF2-deficient cells, nanoparticles?)

Transduction efficiency and selectivity

- Intratumoral delivery *vs*. Systemic delivery (CSF?)
- NF2 expression levels and expression mosaicism for efficacy

Safety of acute over-(re-)expression of *NF2*:

- Potential dominant negative/dominant effect of *NF2 isoforms* overexpression
- Effect on normal ($NF2^{+/-}$) cells?
- Selectivity (tissue-specific promoters?)
- Safety

Delivery Strategies for NF2/SWN Gene Therapy to the PNS and Meninges

Wide spread delivery in the brain and spinal cord can be achieved through the CSF:
- intracerebroventricular (ICV)
- intracisterna magna
- intrathecal injections

(Hocquemiller et al., 2016; Taghain et al., 2020)

Intrathecal injections by lumbar puncture achieve extensive spinal cord transduction

Administration into the cisterna magna delivers the drug closer to the targeted brain areas and has shown transduction in the spinal cord as well as brain

CSF injection of AAV vectors deliver genes throughout the brain and spinal cord in non-human primates
(Bey et al., 2020)

Gene replacement therapy in a schwannoma mouse model of NF2

Shilpa Prabhakar (X. Breakefield Lab.) and Roberta L. Beauchamp (V. Ramesh Lab.) et Al.

Submitted for publication

- AAV-based delivery of functional merlin induces inhibition of mTORC1 activation in NF2-null AC and SC cells in culture.
- A single intratumoral injection of an AAV-merlin vector suppressed tumor growth in a sciatic nerve xenograft model using human NF2-null immortalized Schwann cells
- The study serve as a proof of principle that restored merlin expression in NF2-deficient tumor cells can provide therapeutic efficacy

Summary

- Viral and macro-therapeutic delivery are potential options for *Nf2* gene replacement in *NF2*-mutant tumors
- These approaches initiate a new pipeline for preclinical studies in NF2/schwannomatosis
- Advanced testing of these approaches (safety/efficacy) will require scaling up in larger animal models