

HOE FUNDAMENTEEL ONDERZOEK KAN LEIDEN TOT EEN BK VIRUS THERAPIE

BENELUX Kidney Meeting 2019

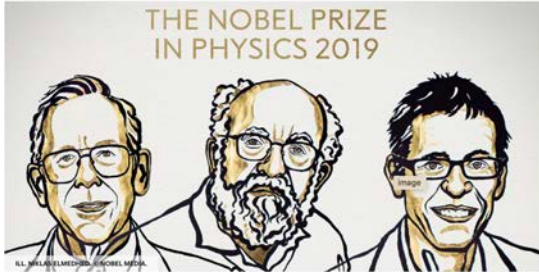


HYBRIDIZE
PHARMA

Disclosure

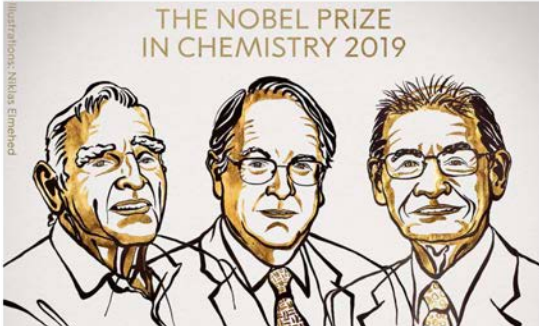
Ik ben (mede-)oprichter, en CEO van Hybridize Pharma.
Ik ben aandeelhouder van Hybridize Pharma.





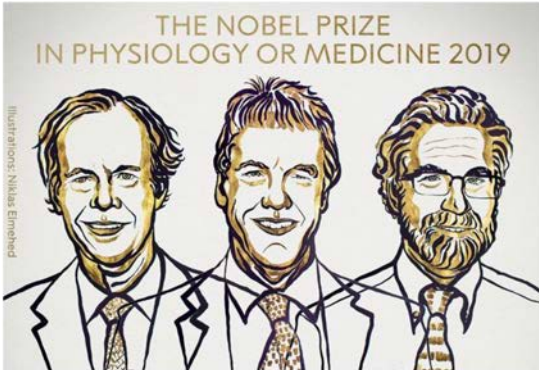
Nobel Prijs voor Natuurkunde

James Peebles – Princeton University (USA)
Michael Mayor – University of Geneva (Switzerland)
Didier Queloz – University of Cambridge (UK)



Nobel Prijs voor Scheikunde

John B. Goodenough – University of Texas at Austin (USA)
M. Stanley Whittingham – Binghampton University (USA)
Akahira Yoshino – Asahi Kasei Corporation (Japan)



Nobel Prijs in Fysiologie en Geneeskunde

Gregg Semenza – Johns Hopkins University (USA)
Sir Peter Ratcliffe – Oxford University (UK)
William Kaelin Jr. – Harvard University (USA)

Wie ben ik en hoe kom ik hier terecht?



Ajax, Ontario, Canada



Windsor en Guelph, Ontario, Canada



Amsterdam (M.Sc)



London, Ontario, Canada (Ph.D)



LACDR, Leiden



LUMC, Leiden

Mijn wetenschappelijke carrière in een notendop



Pre-B-Cell Colony-Enhancing Factor Regulates NAD⁺-Dependent Protein Deacetylase Activity and Promotes Vascular Smooth Muscle Cell Maturation

Eric van der Vliet, Zengxian Nong, Caroline O'Neil, Brad Vengelen, David Freeman, J. Geoffrey Pickering

[illegible][illegible]

ACCELERATED PUBLICATION

Extension of Human Cell Lifespan by Nicotinamide Phosphoribosyltransferase¹

© 1999 Blackwell Science Ltd, *Journal of Internal Medicine* 245: 365–372

the fact that the average longevity (median 82.1) and the average age at death (median 82.9) were not significantly lower for SERT1 carriers than for SERT2 carriers (Fig. 1). However, we also included the fraction of life that was accumulated after age 70 in the regression model. In this model, SERT1 carriers were again found to have a significantly lower life expectancy, and increased the rate of life degradation after age 70 (Fig. 2). This result is consistent with the age-specific effects of SERT2. These data indicate that SERT1 carriers have a lower life expectancy because they start off stress resistance 100–120 h in expressing SERT1-mediated life degradation.

This paper is available online at www.blackwell-sydney.com

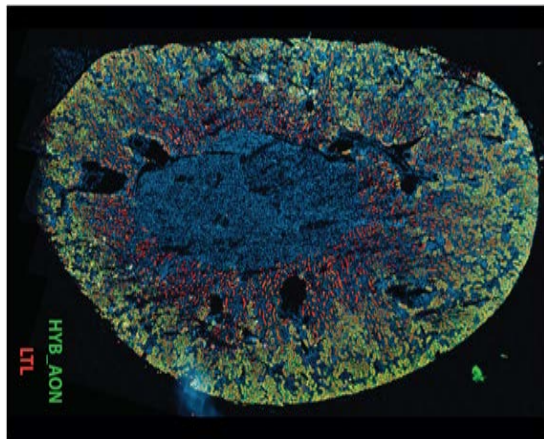
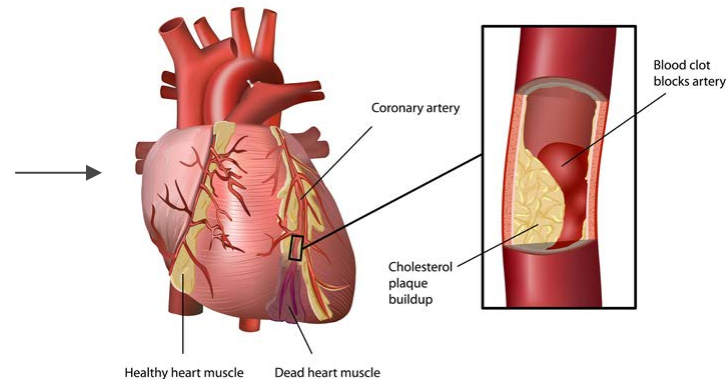
study revealed the arterial wall. Vascular smooth muscle cells (VSMC) are especially important in this regard: the efflux of which VLDL's inhibit a developing atherosclerosis, how dangerous whether the lesion will regress, a potentially to avoid. Strategies to prevent the progression of atherosclerosis of VLDL could be a promising approach for reducing vascular disease.

Nicotinic acetylcholine receptor (nAChR) subunit expression in Pdx-1 null cells (data not shown) and Valentin *et al.* (2000) have shown that the NAC⁺ neurosecretory phenotype is maintained in the absence of Pdx-1 and is not dependent on the expression of the *choline acetyltransferase* (*ChAT*) gene. This is consistent with the idea that the NAC⁺ phenotype is maintained in the absence of Pdx-1, but that the NAC⁺ phenotype is not dependent on the expression of the *ChAT* gene. This is consistent with the idea that the NAC⁺ phenotype is maintained in the absence of Pdx-1, but that the NAC⁺ phenotype is not dependent on the expression of the *ChAT* gene.

Cell Culture—Erythrocytes were gelatinized using prime liquid media (M400) (Becton Dickinson) and erythrocytes sedimented through water and used for the RBC-DA assay. Blood from sheep, especially generated from the same flock, was used as the erythrocyte source. The RBC-DA assay was performed using fresh erythrocytes from sheep obtained from the United Kingdom.

To quantify erythrocyte loss, sheep were placed at a 500 mL water trough and 100 mL of water was changed every 4 days and sheep received 0.5% sodium chloride. Harvested cells were counted from single aliquots and the number of erythrocyte findings was determined based on $\text{g} \times \text{cm}^{-3}$ (mean $\pm$ SD) (Fig. 1). Erythrocyte loss was analyzed daily for 14 days. Population growth curves were compared using non-linear regression.

Recombinant Erythropoietin and Erythropoietin Receptor—A central gene delivery system was used to generate human erythropoietin (EPO) and erythropoietin receptor (EPO-R) (Genzyme Corporation, Boston, MA, USA). Recombinant erythropoietin (EPO) (Genzyme, Boston, MA, USA) was used for the RBC-DA assay.

 Springer

Emerging roles for RNA-binding proteins as effectors and regulators of cardiovascular disease

Robert G. de Bruin^{1,2}, Ton J. Rabenold^{1,2}, Anton Jan van Zonneveld^{1,2}, and Eric F. van der Weer^{1,3,4}

The relationship between cognitive ageing and health has proven to be complex in relation to the role of cognitive decline in the development of dementia and other age-related diseases. Translational and population-based approaches have led to the identification of neurobiological correlates of cognitive decline, but the mechanisms of neurobiological correlates of cognitive decline are still unclear. The identification of neurobiological correlates of cognitive decline is a key step in the development of interventions to delay or prevent cognitive decline. This review discusses the current state of knowledge on the neurobiological correlates of cognitive decline, with a focus on the role of the hippocampus in memory and learning. The review also discusses the role of the hippocampus in other cognitive functions, such as executive function and attention. The review concludes by discussing the implications of the findings for the development of interventions to delay or prevent cognitive decline.

along a 100-m segment.

Introduction

The regulation of cellular phenotype is intimately connected with the expression of specific genes and the establishment of a cell's epigenetic state. The cell's epigenetic state is defined by the methylation of cytosine (CpG) sites in the genome [1,2]. CpG methylation is a reversible process that can be regulated by a variety of factors, including DNA methyltransferases and demethylases. The methylation of CpG sites is a key regulatory mechanism for gene expression, and it is involved in many biological processes, including development, differentiation, and disease. In this review, we will discuss the role of CpG methylation in cellular phenotype regulation and the factors that influence its expression.

ARTICLE
Received 17 Jul 2015; accepted 26 Jan 2016; published 2 Feb 2016
DOI: 10.1126/science.1261000
Quaking promotes monocyte differentiation

Robert G. de Boer^{1,2}, Lily Sheng², Jansen Pineda², Kelly C. de Boer², Arjuna Singh², W. Samuel

[illegible]

Abstracts are only available in English. For more information, visit <http://www.elsevier.com/locate/bsc>.

[illegible]

SCIENTIFICO PER PEO

OPEN The RNA-binding protein quaking maintains endothelial barrier

function and affects VE-cadherin and β -catenin protein expression

Protein regulation of endothelial cell contractility is essential for phenomena of endothelial cell proliferation. Interestingly, these processes are actively involved in the control of vascular leakage. Endothelial dysfunction, and the initiation and progression of atherosclerosis. We found that the endothelial binding of protein gelsolin is tightly regulated by endothelial cells, and that its expression was augmented by proteolytic cleavage under shear flow and the transcription factor NF- κ B binding to the promoter. Moreover, we demonstrated that gelsolin binds to the cellular contractile apparatus and can reduce cellular tension mediated by the F- α 2H of α -actinin. Reduced gelsolin levels attenuated VEGF-induced and α -actinin-regulated endothelial cell function in vitro and resulted in increased endothelial-induced vascular leakage in vivo. Taken together, we report that gelsolin is a powerful microtubule-binding factor. Our results provide novel insight into the importance of post-translational regulation in controlling vascular integrity.

[illegible]

Copyright Clearance Center

Quaking, an RNA-Binding Protein, Is a Critical Regulator of Vascular Smooth Muscle Cell Phenotype

Jim Wex, Tomer Patai, Hil M. Siegel, Stella M. Siegel, Janice M. van Gils, Marko K. Borsari,
Craig M. Buckner, Peter L. van Sandt, Andrew Anderson, Craig van Sandt, Jim Vanhook,
Henry C. de Boer, Eric A. Francis, Roel Bijkert, Ma Renssch, Mirya Dango, John Kasper,
Marion Jan Schell, Alfred C. van der Wal, Stephane Richard, Tom E.C. van Boven
J. Geoffrey Pickering, Peter S. Hoonen, Marie-Jean Guayrou, Tom J. Kohnen, Janine M.J. de Vos

Rationale: RNA-binding proteins are critical post-transcriptional regulators of RNA and can influence gene expression, RNA localization, and stability. The RNA-binding protein Quaking (QKI) is essential for embryonic blood vessel development. However, the role of QKI in the adult vasculature, and in particular in vascular smooth muscle cells (VSMCs), is currently unknown.

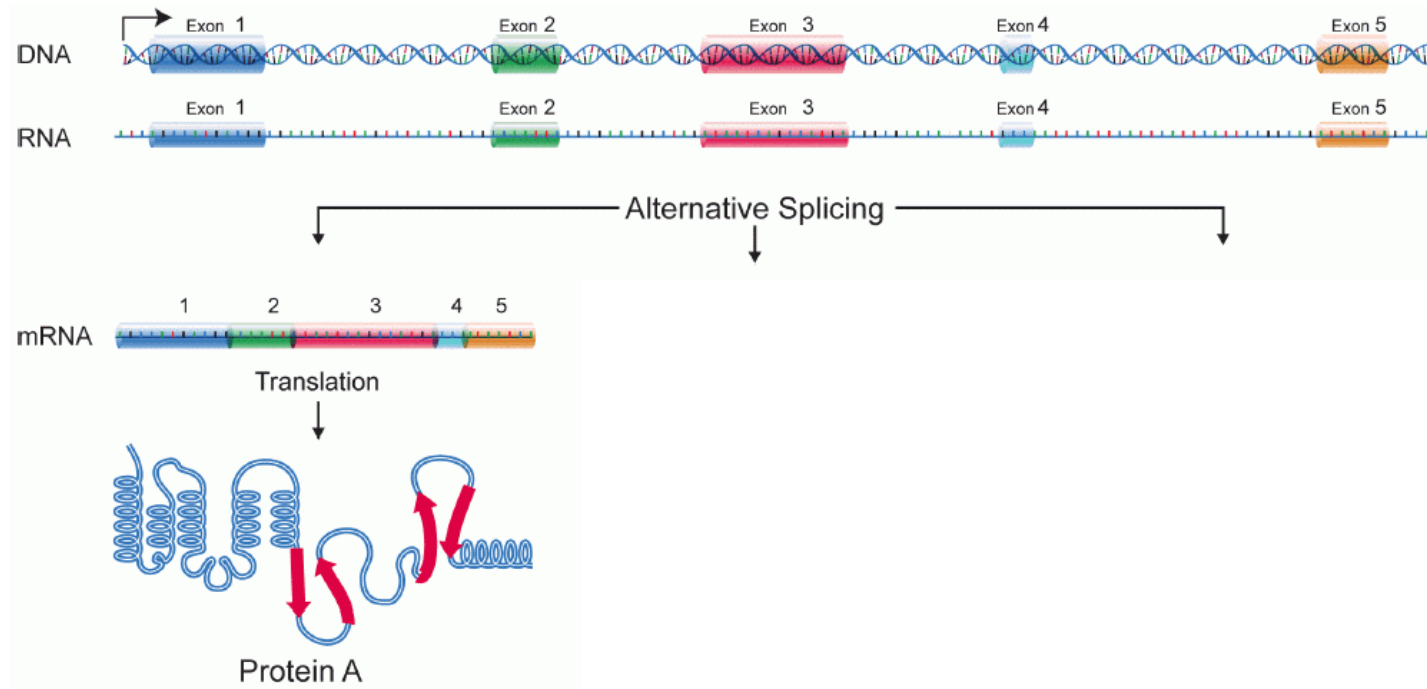
severe lesions, but not acutely toxic, in a mouse model of cocaine injury, on chemical-induced neuronal apoptosis in Quaking null mice, which have decreased DAT expression. Conversely, alteration of DAT increased hippocampal apoptosis in *Quaking*^{+/+} mice, while protein levels of another separate gene, *cytochrome c*, remained unchanged. *Quaking*^{+/+} mice also had a 100% reduction in DAT levels in the hippocampus, whereas 20% reduction in the neocortex (see below) and explained the uplink of abnormal mice. To gain transcriptional cross support the *Quaking*^{+/+} and *Quaking*^{0/0} mice were also analyzed by microarray. The quaking transcription factor is one of 34 genes, transcriptionally downregulated that showed similar expression patterns, including one of the genes, *cytochrome c*, which is also downregulated in *Quaking*^{0/0} mice. Conclusions: We propose that DAT is a critical regulator of 1500+ genes phenotypically and that loss-function in *Quaking* can contribute to cocaine-induced neurodegeneration in cocaine injury. (Cite this: 2003.03.0005.0025)

Real Time differential expression • *cytochrome c* • *Quaking* • *Ct* • *cytochrome c* • RNA-protein complex (Quaking)

D As trading proceeds, the market becomes more liquid, and the bid-ask spread narrows. The bid-ask spread is the difference between the highest price a buyer is willing to pay (the bid) and the lowest price a seller is willing to accept (the ask).

[illegible]

Wat is alternative splicing?



Hoe werkt alternatieve splicing nou echt?



A



Verbeterd functie

B



Geen echte verandering

C

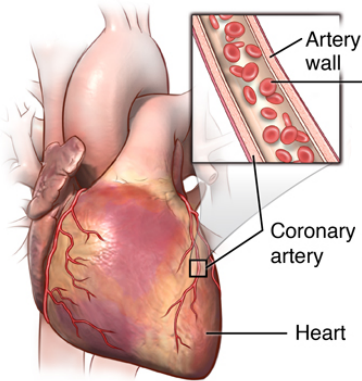


Verminderd functie

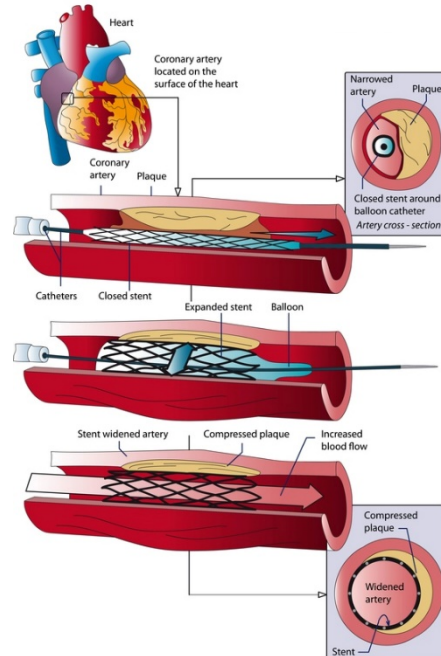
D



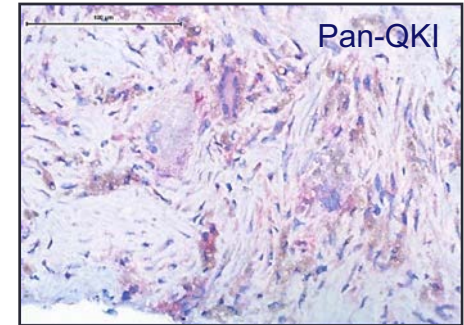
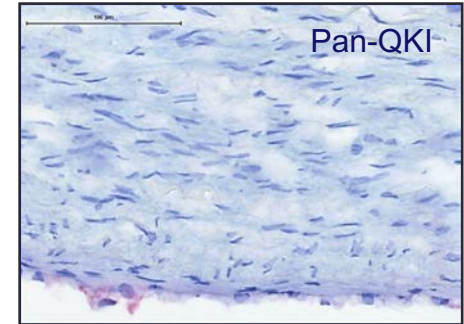
Stents om nieuwe vernauwing te voorkomen



Percutane Coronaire Interventie (Dotteren)

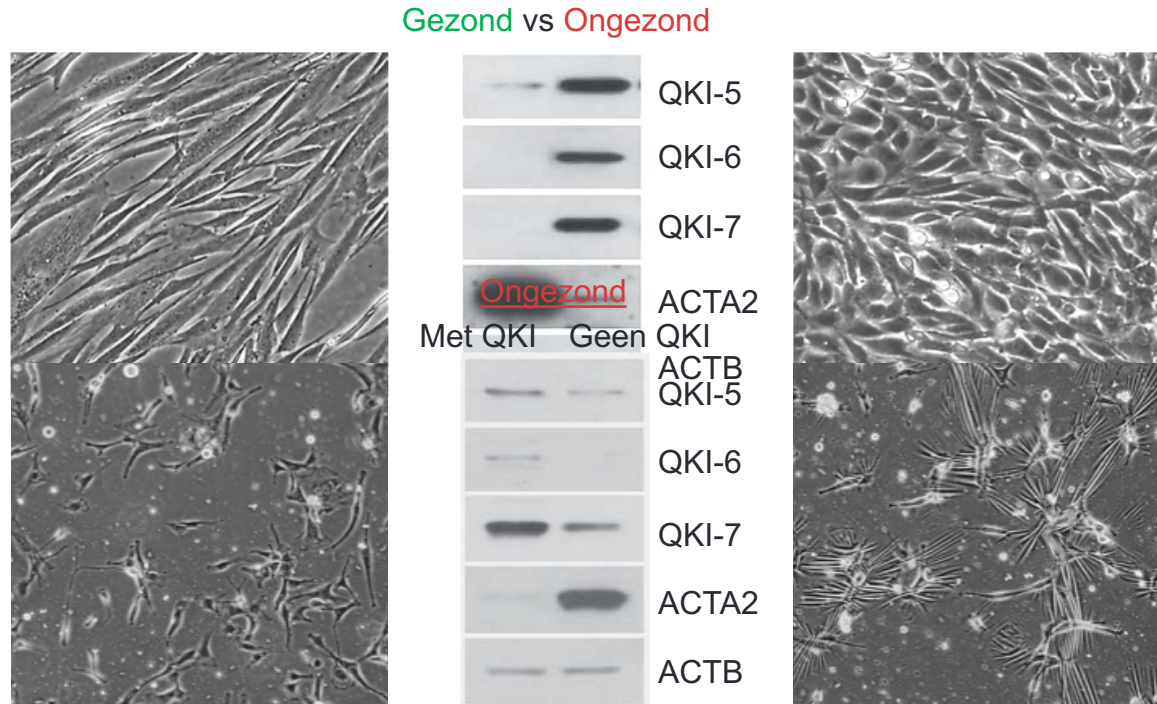


Gezonde arterie

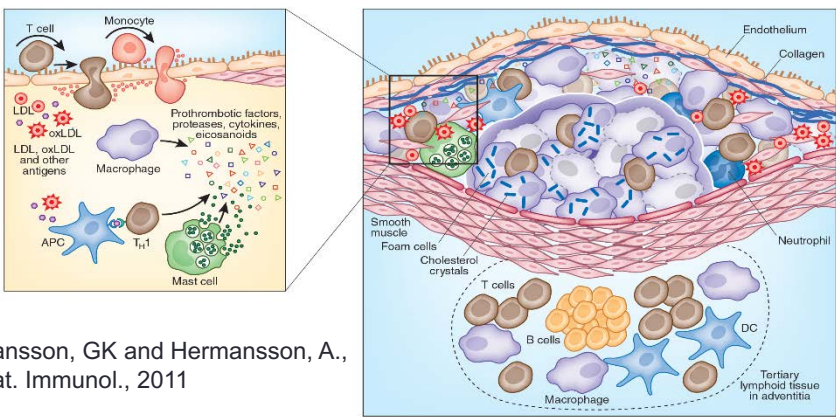


Ongezonde arterie

Door splicing to moduleren kan je ongezonde cellen weer gezond maken

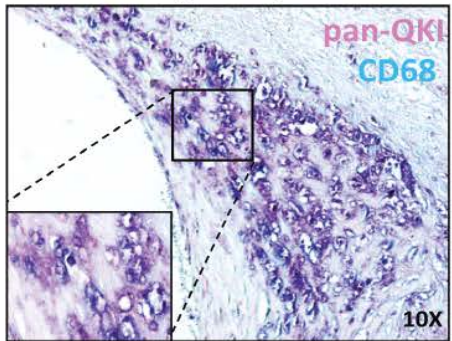


Aderverkalking – chronische ontsteking van de vaten

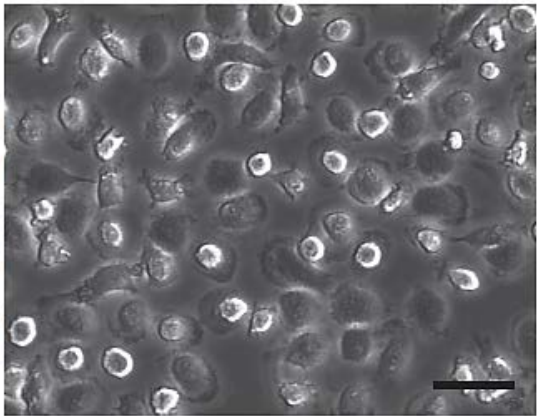


Hansson, GK and Hermansson, A.,
Nat. Immunol., 2011

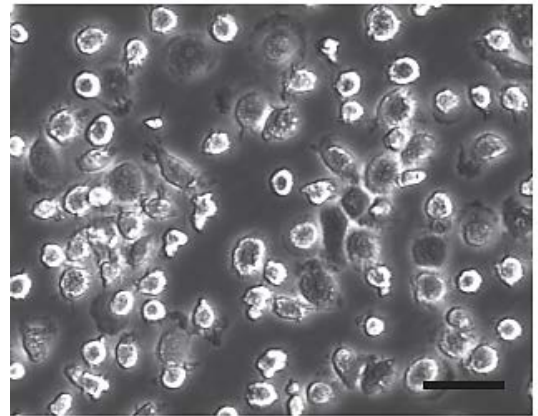
Bloedvat met
aderverkalking



Zus met
normale
hoeveelheid
Quaking

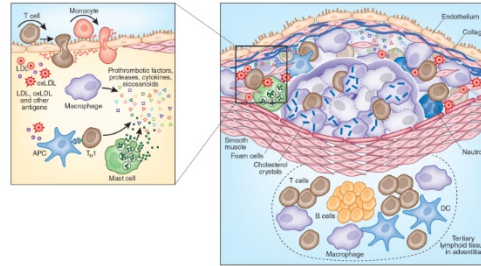


Patient met
minder Quaking



Gezond (monocyt)

QKI laag

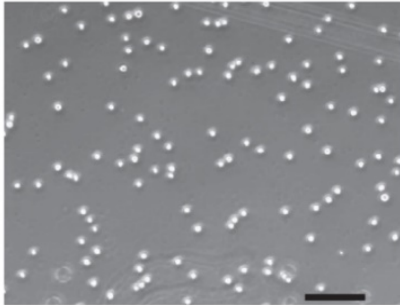


Ongezonder (macrofaag)

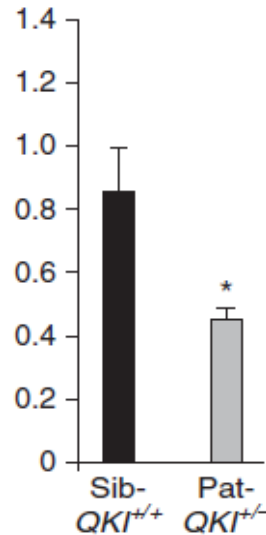
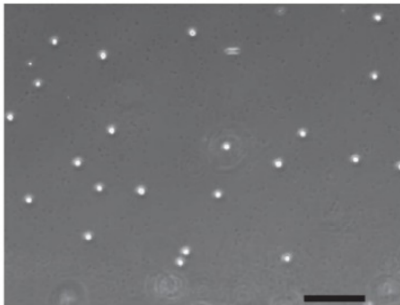
QKI hoog

Adhesie

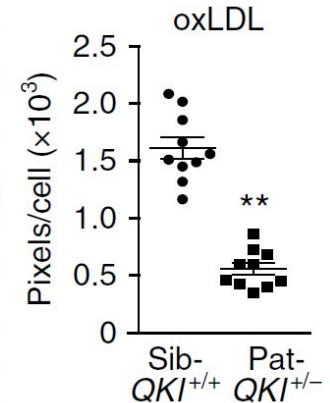
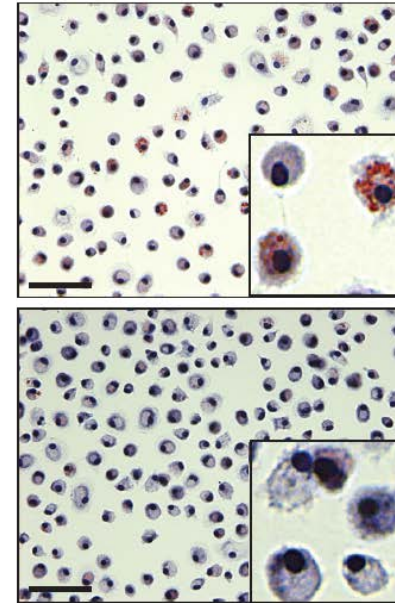
Normaal
Quaking



Minder
Quaking



Vet opname



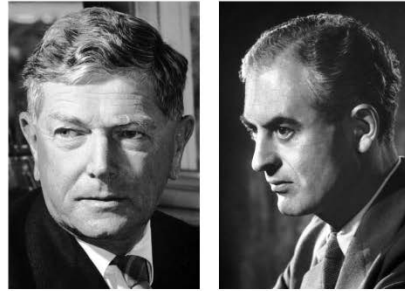
Orgaan transplantatie: Hoe denkt het Nobel committee daarover?

1911



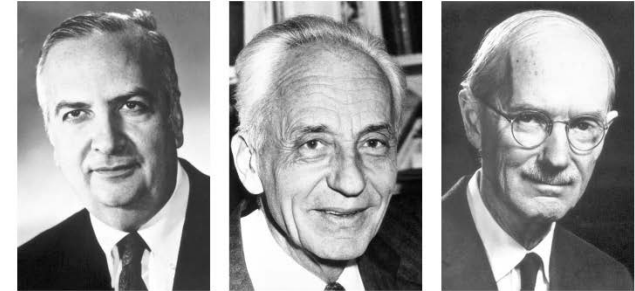
Alexis Carell

1960



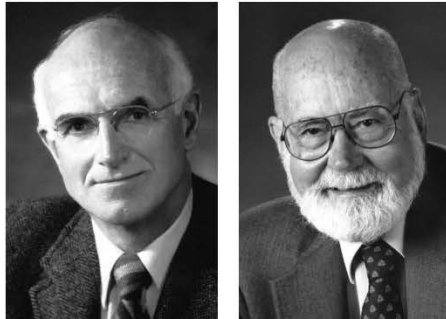
Macfarlane Burnet & Medawar

1980



Benacerraf, Dausset & Snell

1990



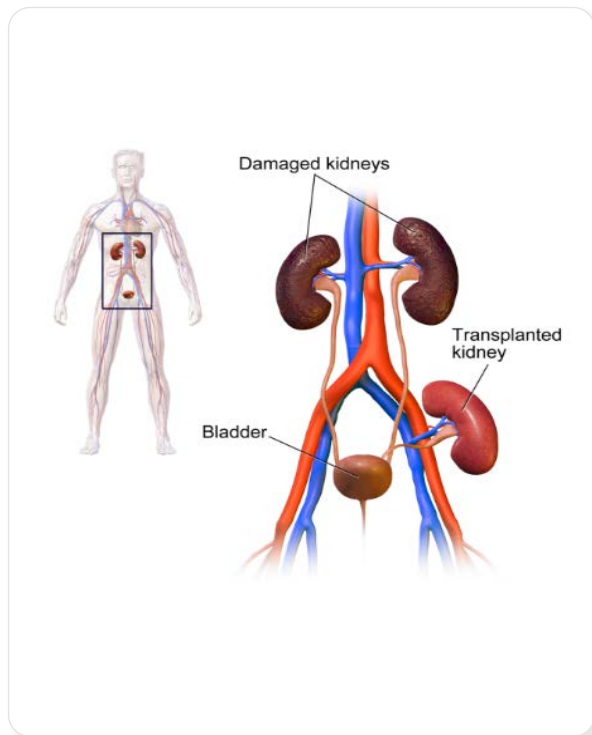
Murray & Thomas

1996



Doherty & Zinkernagel

Niertransplantatie: de beste oplossing voor patienten met nierinsufficiëntie



- 2.6 miljoen met eindstadium nierfalen wereldwijd

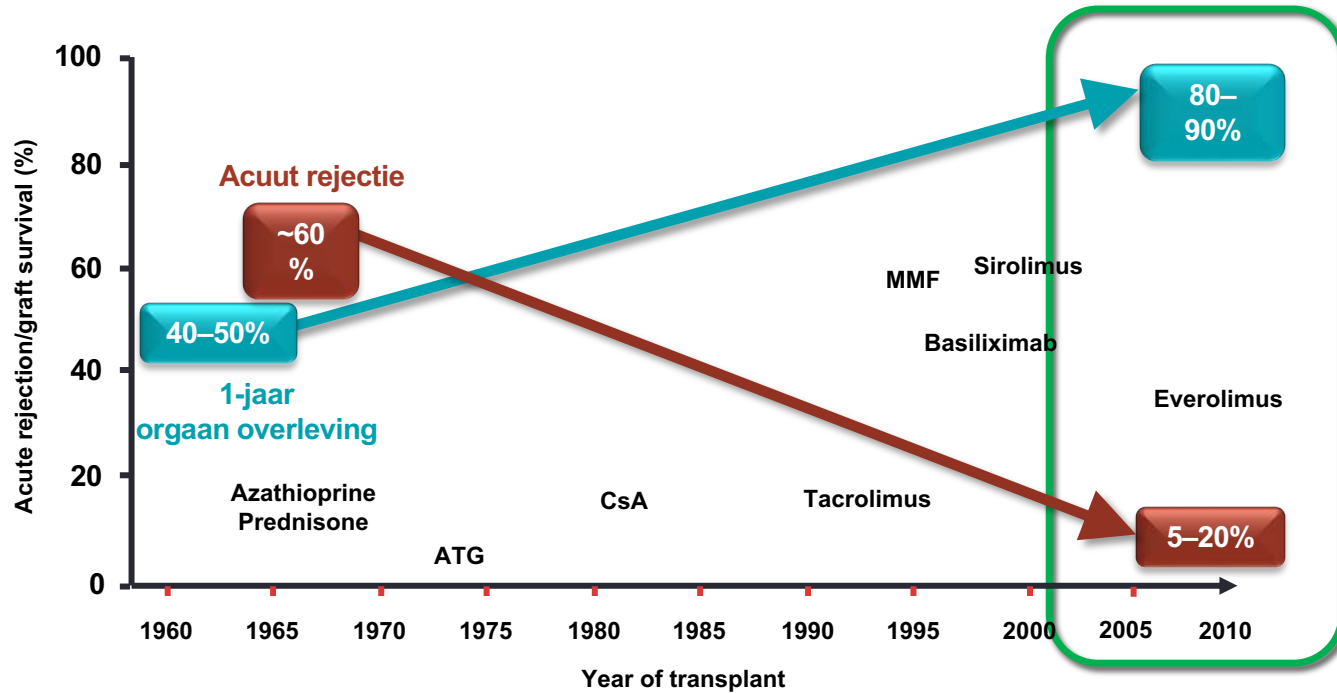
Risico factoren: Leeftijd, Genetisch, Alcohol, Zuckerziekte*,
Obesitas* (* toenemend in frequency)

- Nier-vervangend therapie mogelijkheden

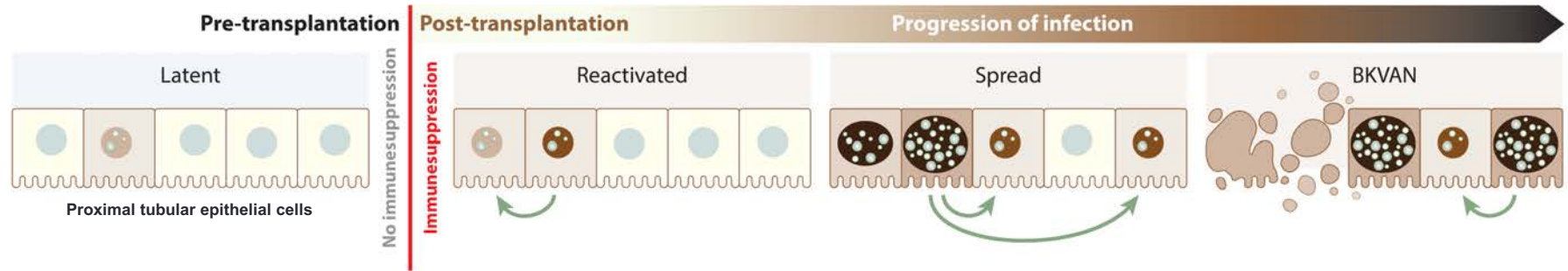
- | | | |
|-----------------------|---|--|
| 1) Hemodialyse | } | 5-10 jaar max. |
| 2) Peritoneal dialyse | | |
| 3) Niertransplantatie | } | Meest aantrekkelijk optie
Gem. Wachtijd: 679 days |

- 135,860 orgaan transplantaties in 2017 wereldwijd; 89,823 nier transplantaties (66%)

Niertransplantatie: Inmiddels een succes verhaal?

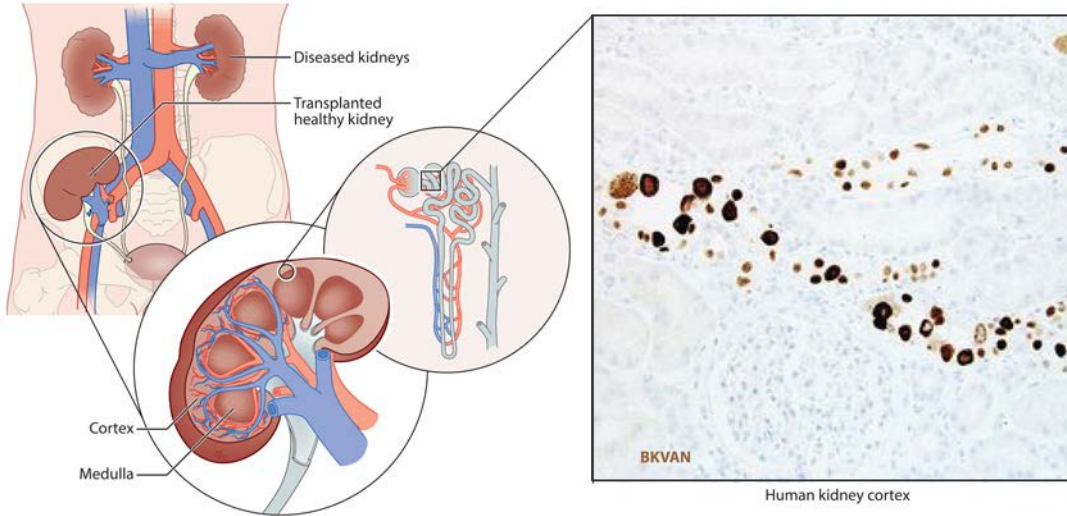


BKVAN: Immuunsuppressie-geïnduceerd nierschade



- Immuunsuppressie kan leiden tot BK virus reactivatie (uit een soort van sluimerstand)
- Het produceren van virus in deze cellen leidt tot het opzwellen van de kernen en het uitscheiden van nieuwe virus partikels die ervoor zorgen dat buurcellen ook geïnfecteerd raken
- Tubulair epitheel cellen gaan dood, waardoor nierfunctie en levensduur achteruit gaan

BK virus: Een virus dat nierfunctie en levensduur ernstig kan verminderen



Er bestaat geen therapie!

- Bij ~40% van niertransplantatie patienten zal BK virus gedetecteerd worden in hun bloed
- ~10% van deze patienten zullen ernstig schade oplopen aan hun nier (dit heet BK virus-geassocieerde nefropathie), en bij ~5% kan dit zelfs leiden tot verlies van hun nieuwe nier
- Dit kan de levensduur van de nieuwe nier met ~2-3 jaar verminderen

Orgaan transplantatie: Hoe denkt het Nobel committee daarover?

1911



Alexis Carell

1960



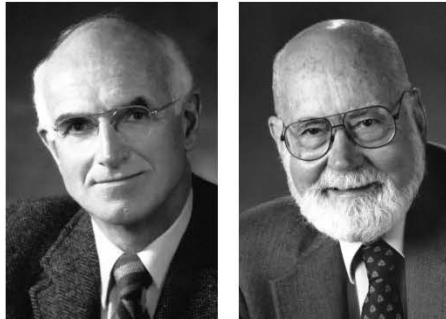
Macfarlane Burnet & Medawar

1980



Benacerraf, Dausset & Snell

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Murray & Thomas

1996

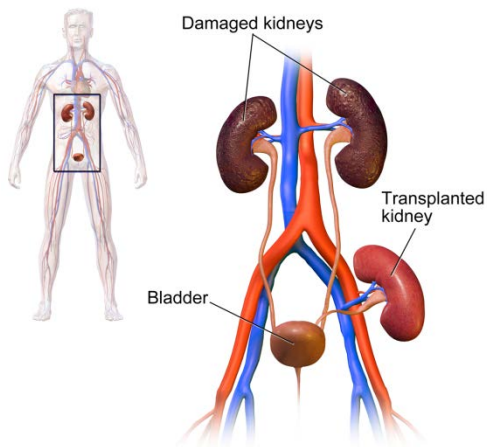


Doherty & Zinkernagel



BK virus in niertransplantatie: Een probleem zonder oplossing

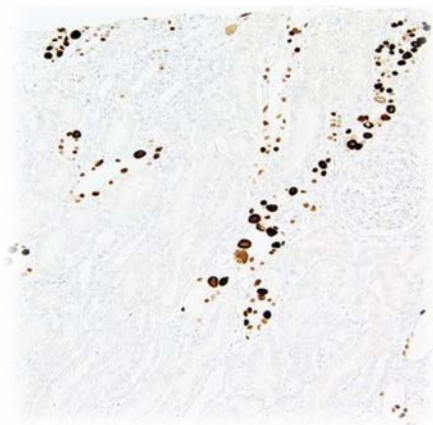
Niertransplantatie



~ 85,000 patiënten
per jaar



BK virus



30-40% lopen risico op
BK virus-gemedieerd
nierschade



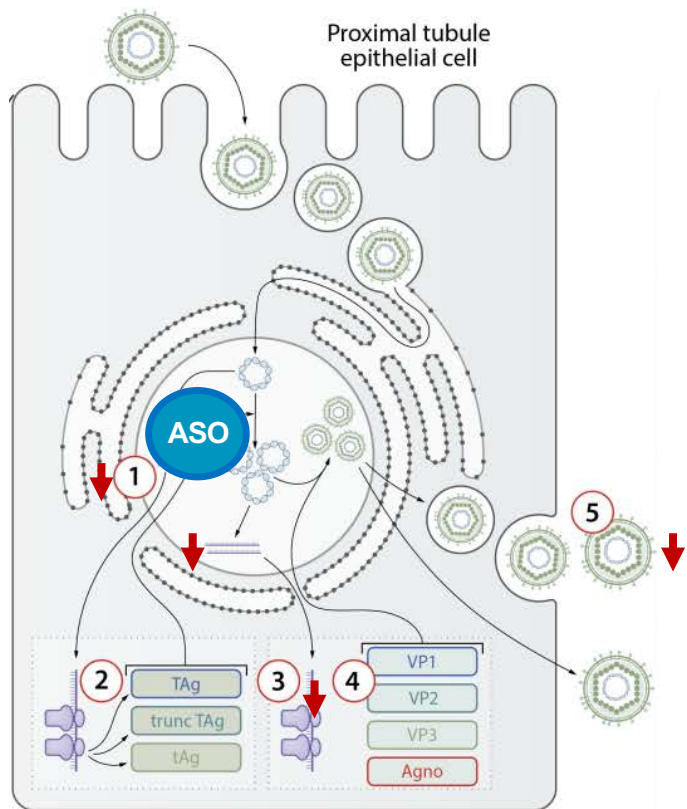
Onze oplossing



Innovatieve BK-specifiek
antisense oligonucleotide

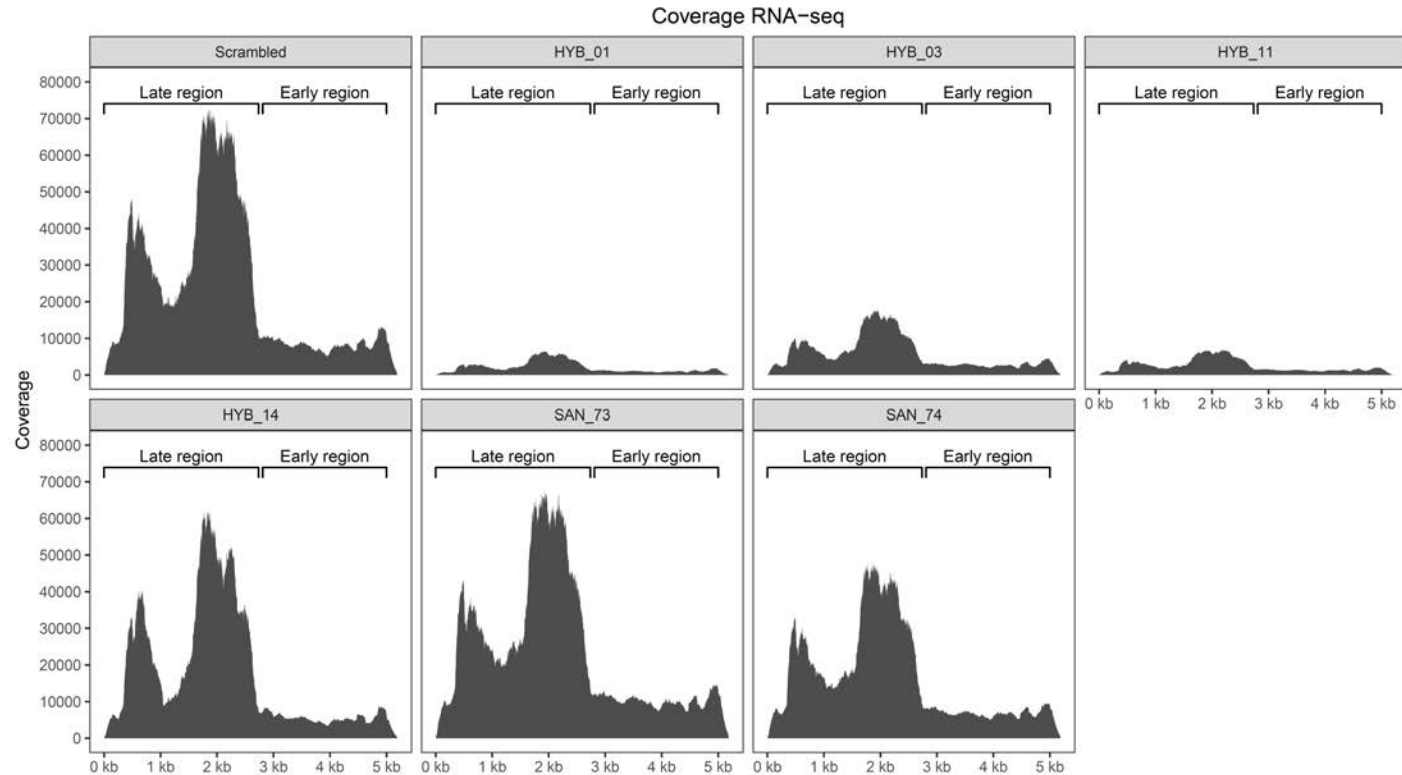


Hoe weten we of ons geneesmiddel wel of niet goed werkt?

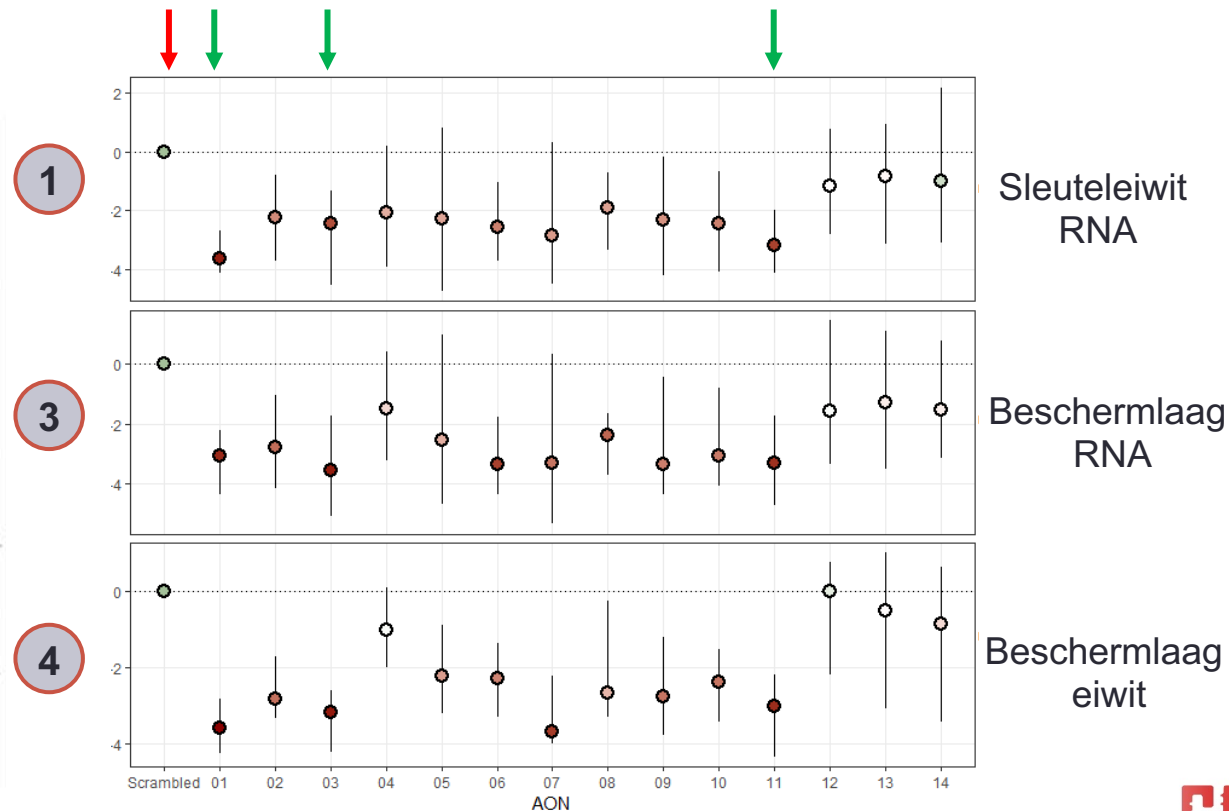
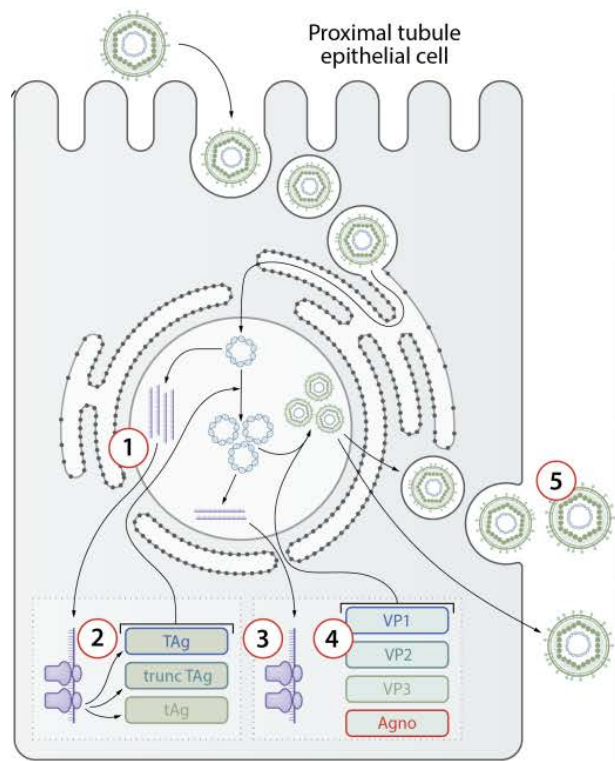


1. **Reductie** van RNA van sleuteleiwit
2. **Reductie** van RNA dat nodig is om het virus te voorzien van een beschermlaag
3. **Reductie** van beschermlaag eiwit
4. **Reductie** van virus productie of capaciteit om zijn buurcellen ook te infecteren.

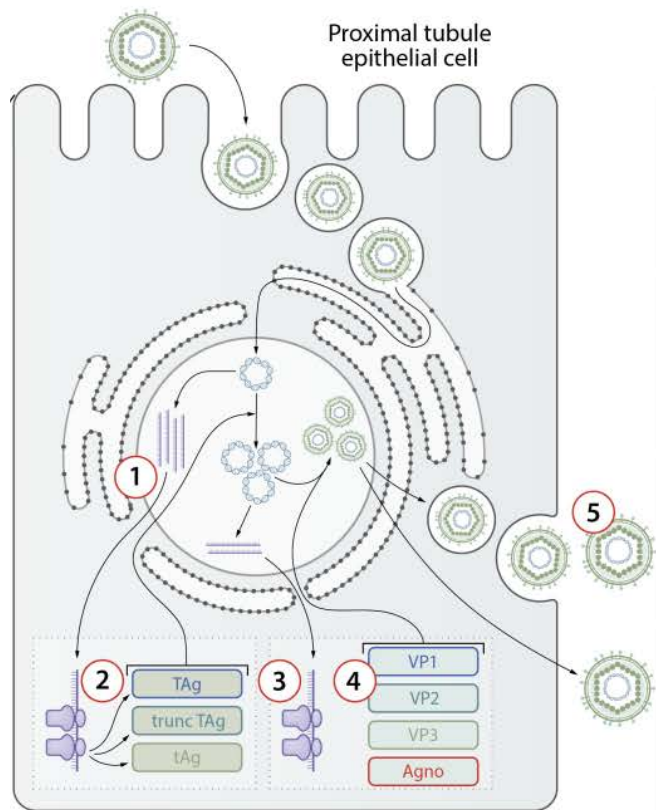
Ons geneesmiddel vermindert productie van BK virus RNAs



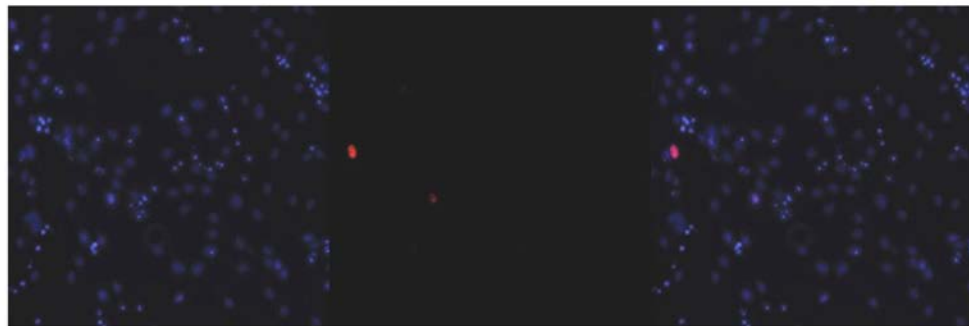
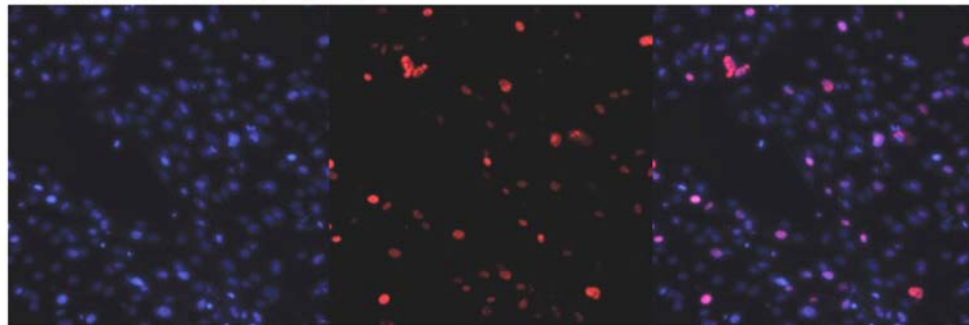
Testen van de effectiviteit van ons geneesmiddel in humane niercellen



Testen van de effectiviteit van ons geneesmiddel in humane niercellen

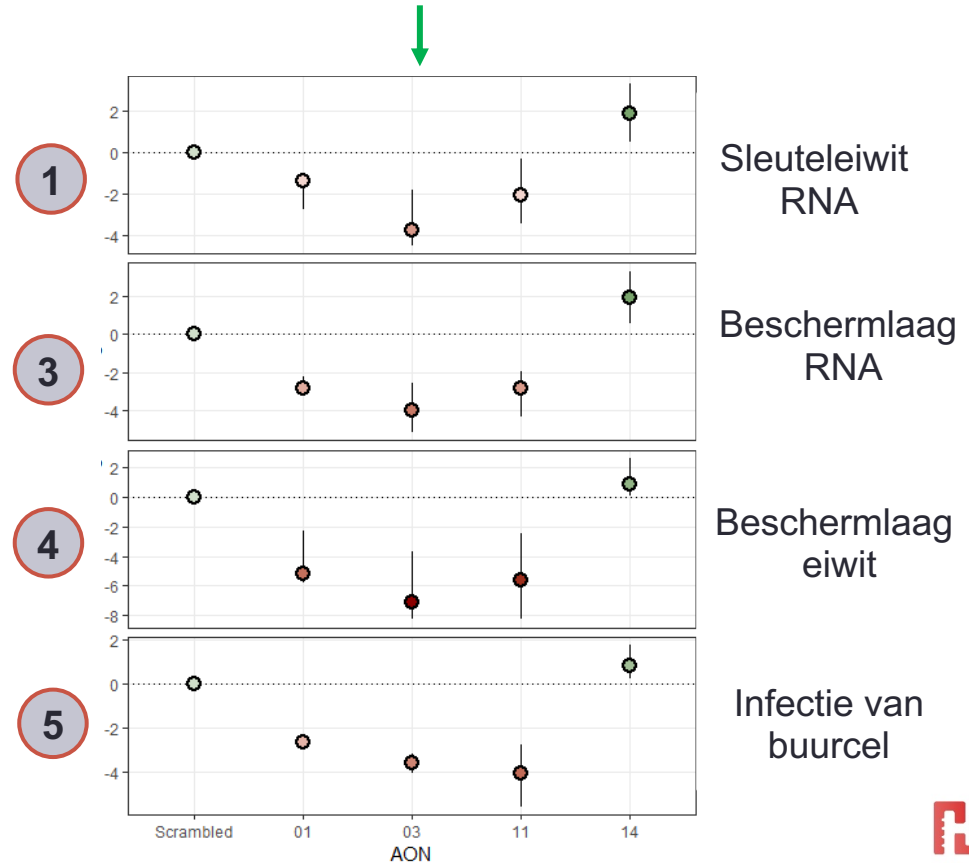
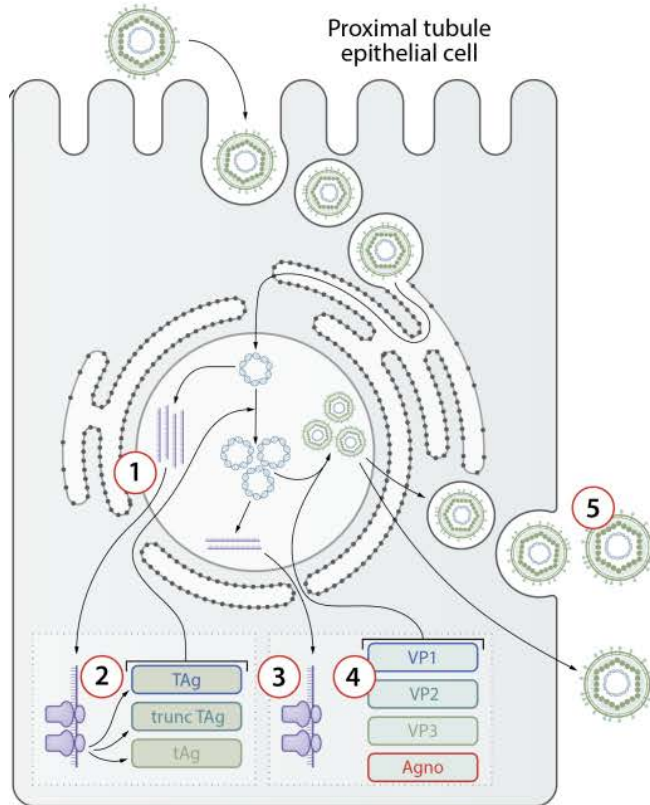


Controle RNA

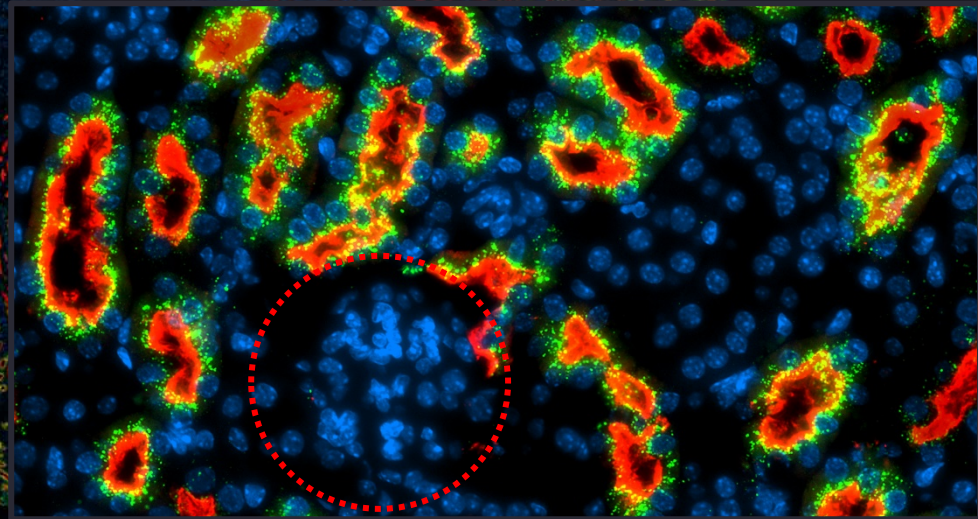
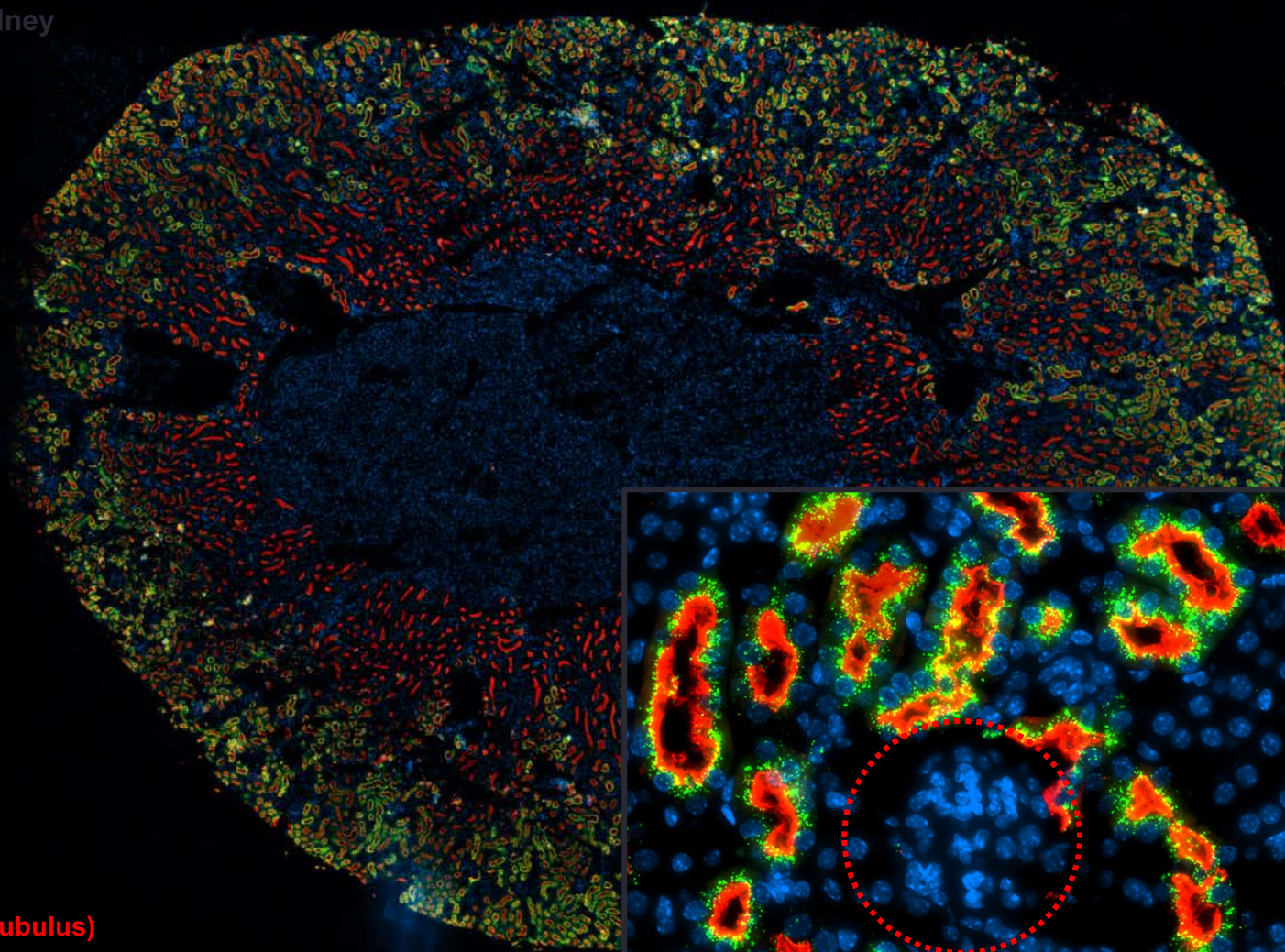


Ons geneesmiddel

Meer testen: nu in niercellen van humaan weefsel

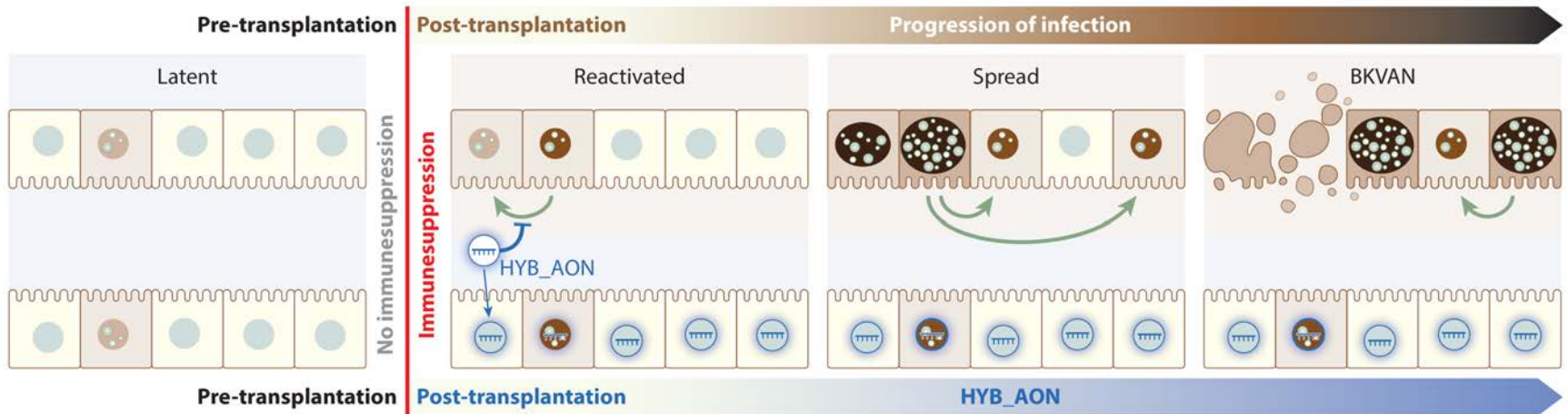


8w C57BL/6 kidney
24h post i.v.
40 mg/kg AON

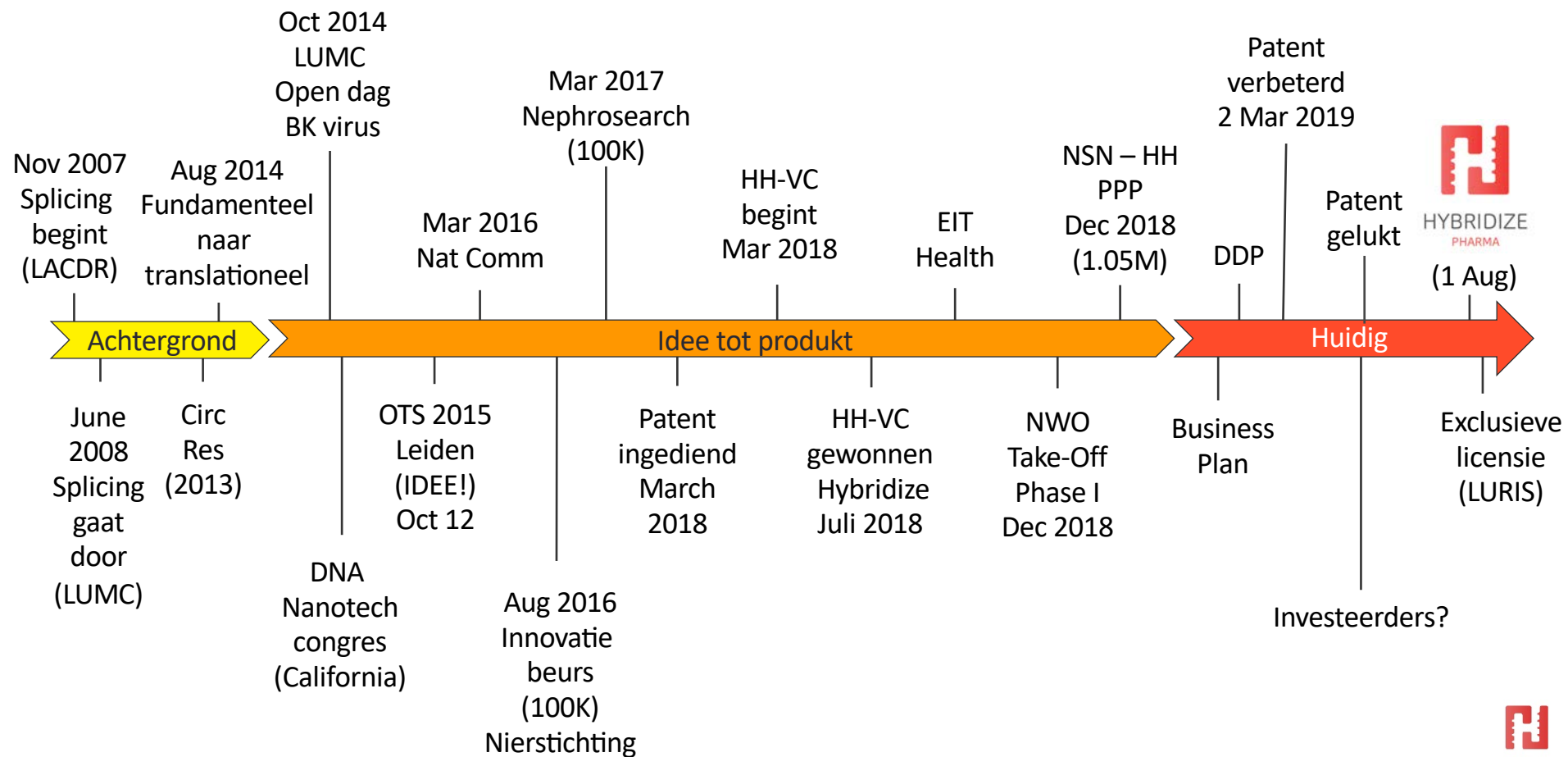


HOECHST
LTL (proximale tubulus)
RNA

Samenvatting



Het werkt in een petrischaaltje? Werkt het ook in een dier/mens?



Acknowledgements

Nephrology, LUMC

- Anton Jan van Zonneveld
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- Carla van Alem

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- Herman Wunderink
- Els van der Meijden

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- Willeke van Roon

SASC, LUMC

- Leon Mei

Universidad de Navarra, San Sebastián, Spain

- Juan Pablo Romero

University of Massachusetts Medical School, USA

- Jonathan Watts

University of North Carolina School of Medicine, USA

- Volker Nickeleit

