I have no **conflicts of interest** to disclose.
Growing tissue stem cells into organoids and tumoroids

Hans Clevers. Hubrecht Institute, Utrecht, Holland
A conveyor belt renews the tissue every 4 days
Lgr5 is an unusual Wnt target gene
It Marks Cycling Crypt Base Columnar Cells.
(Nick Barker)
Charles Philippe Leblond (1910 – 2007)
Stem cell pioneer

2004: “A month ago, I thought Power Point was a tool for sharpening pencils.”
Lgr5-Driven GFP in Crypt Base Columnar Cells
Rosa-Confetti
Multicolor lineage tracing based on Brainbow (J. Lichtman)

Hugo Snippert
Laurens van der Flier
Ben Simons
(Cambridge)
Fifteen Lgr5 stem cells in each crypt divide every day. They neutrally compete for space.
Lgr5+ cells are Intestinal Stem Cells
Lgr5 is the receptor for R-spondin. R-spondin amplifies Wnt signals.
Can we grow single adult Lgr5 cells in vitro?
Sorted Lgr5-driven GFP+ cells from intestine.

Gene expression profiling (Marc van de Wetering)
Single Lgr5 cells form miniguts in 3D-culture
- R-spondin1 (Wnt agonist)
- EGF
- Noggin (BMP inhibitor)
- Matrigel

Toshiro Sato
Het zuiveren en kweken van lichtgevende stamcellen.
LGR5+ cells after 3 passages

A mini-gut self-organizes

Courtesy Inke Nathke
Can we transplant mini-guts grown from a single adult Lgr5 stem cell?
Transplanting the offspring of a single mouse colon stem cell.
(with Tetsuya Nakamura, Shiro Yui and Mamoru Watanabe)
Transplantation of colon miniguts grown from a single stem cell (with Mamoru Watanabe lab)
Long-term survival of transplanted colon organoids grown from a single stem cell.
Transplanted region looks entirely normal.
- Unimpeded expansion from single Lgr5 stem cells.
- Works for human and mouse.
- Stomach, small intestine, colon, liver, pancreas, prostate, lung.
- Organoids are genetically normal and can be transplanted.
Organoid technology for disease modeling

Cystic fibrosis: CFTR, a chloride channel, is defective (with Florijn Dekkers and Jeff Beekman)

Inactive

Active

+Forskolin
Repair of $CFTR-\Delta F508$ in a single colon stem cell from a Cystic Fibrosis Patient: CRISPR/Cas9-enhanced homologous recombination
Wt human CFTR opened with forskolin
Functional correction of CFTR in clonal colon stem cells of cystic fibrosis patients
Using organoids for disease modeling

Growing organoids from colon, pancreas and prostate cancer patients
A 'living' biobank

Sta Op Tegen Kanker Dreamteam
25 pairs of wt and colorectal cancer organoids grown to date. Tumoroids are always Wnt-independent. (with Matthew Meyerson & Mike Stratton)
Colorectal cancer organoids represent primary tumors

Patient 6

Patient 8

Patient 9

Patient 11

Patient 14

Primary tumor

Organoid
Mutation frequencies in tumor organoids
Genes significantly mutated in colorectal cancer

- APC
- P53
- KRAS
- PIK3CA
- FBXW7
- SMAD4
- TCF7L2
- BRAF
- NRAS
- ARID1A
- ARID2
- ACVR1B
- ERBB3
- AXIN2
- SMAD2
- CDC27
- CTNNB1
- POLE
Tumor organoids  Normal organoids

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Mesenchymal type

Epithelial type

Andreas Schlicker
Lodewyk Wessels
NKI, Amsterdam
The Netherlands
Differential Drug Sensitivity of Colorectal Cancer Organoids

No genetic indicator for 5-FU sensitivity
Organoids may predict drug efficacy
Establishment of Pancreas Organoids from Pancreatic Ducts or Cancers
Human Pancreas Organoids from Pancreas Ductal AdenoCarcinoma (PDAC) or Ampullary Tumor (AT)

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<th>PDAC#T1</th>
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- **E Passage**
- **L Passage**
- **H&E**
- **H&E tissue**
Orthotopic xenografts of T1 and T5 samples.
Prostate organoid culture initiation
with Sawyers Lab (Karthaus et al, submitted)
Human prostate cancer organoids
Gao et al, submitted
Nick Barker
Johan van Es
Marc van de Wetering
Bon-Kyoung Koo
Toshi Sato
Sylvia Boj
Meri Huch
Arnout Schepers
Wim de Lau

Peter Peters, Madelon Maurice, Albert Heck labs
Tetsuya Nakamura, Shiro Yui, Mamoru Watanabe (Tokyo)
Florijn Dekkers, Jeff Beekman (Utrecht)
Ben Simons (Cambridge, UK)
Dave Tuveson, Mike Stratton, Matthew Meyerson, Charles Sawyers Labs