The Semi-Synthetic Artemisinin Project
Industrialization of a Synthetic Biology derived product

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Malaria & Artemisinin

- Malaria most prevalent in subsaharan Africa, south and south-east Asia
  - One third of world population living in affected areas
  - In 2012: 219 million cases, 660,000 deaths
- Increasing resistance to « classical » medication
- Artemisia annua known since more than 2000 years in Chinese medicine
- Artemisinin isolated/identified 1972
- **2004**: WHO recommends artemisinin based combination therapies (ACT) as standard therapy for uncomplicated Malaria
- Demand increase from 50 t/a to appr. 200 t/a over last 10 years
Semi-synthetic Artemisinin Project Goals

• Create a complementary source of non-seasonal, high-quality, and affordable artemisinin to supplement the current plant-derived supply.
  • Cycle time reduction from 15 months to 3 months

• Ensure semi-synthetic artemisinin is available to all qualified derivative manufacturers.

• Contribute to stabilizing the price of ACTs to benefit patients and payers.
Project History

2003: First Publication by J. Keasling et al. on the technology
2004: iOWH/Amyris/UC Berkeley Partnership & 1st BMGF grant
2005: Development phase started
2007: Sanofi-Aventis partnership (industrial partner)
2008: Development phase completed
2009: Industrialization started, 2nd BMGF grant
2011: Industrialization completed (first few 100 kgs produced)
2012: Validation on industrial scale, regulatory submissions
2013: Approval from WHO, Production at multi-ton scale
Simpler Sugar

Acetyl-CoA → ERG10
Acetoacetyl-CoA → ERG13
HMG-CoA → tHMGR X2
Mevalonate → ERG12
Mevalonate-P → ERG8
Mevalonate-PP → ERG19
IPP → DMAPP → ERG20
GPP → ERG20
FPP → ERG20
Met → erg9::P<sub>MET3</sub>-ERG9
Squalene → ERG1, 7, 11, 23, 25, 6, 2, 3, 5, 4

Synthetic Biology

Amorphadiene → ADS
AMO/CPR → ADH1
Non-Enzymatic

Artemisinic acid

Purification

Chemical Conversions

Microbially Derived Artemisinin

Oxidation and Ring-Closure

Dihydroartemisinic Acid Ester

Peroxidation

Dihydroartemisinic Acid Ester

Esterification

Dihydroartemisinic Acid

Reduction

Artmisinic acid

Synthetic Biology

Chemical Conversions

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Peroxidation

Dihydroartemisinic Acid Ester

Esterification

Dihydroartemisinic Acid

Reduction

Artmisinic acid
Fermentation Part: Industrialization

In 2008 strains were transferred from Amyris to Sanofi.

- Development to meet industrial strain requirements
  - Reproducibility of different yeast strains
  - Banking (strain stability)
- Optimization of fermentation performance
  - Introduction of ethanol as second carbon source
- Optimization of down-stream process
  - Laboratory process could not be scaled up (foaming)
  - Use of one organic solvent for continuous extraction only (for recycling purpose)
  - Crystallization of product from water
Fermentation Part – Artemisinic Acid Industrial Scale at Huvepharma (Bulgaria)

- Process development and industrial scale-up completed.
- Facility and equipment are in place.
- Routine Production Started
- 2012: 38.7 t produced
- 2013: Production of 60 t targeted
Chemistry Part: Industrialization

In 2008 lab process was transferred from Amyris to Sanofi.

– Hydrogenation step (1)
  • Catalyst changed to a more selective one

– Activation/Esterification step (2)
  • New activation group introduced to facilitate following steps

– Photochemical Oxydation steps (3/4)
  • Process changed completely
  • Photochemical process with concatenation of oxydation and rearrangement reactions
  • Chromatography skipped during work-up

– Overall yield almost tripled (19 % → 55+ %)
Chemistry Part – Semisynthetic Artemisinin Industrial Scale in Garessio (Italy)

- Process development and industrial scale-up completed.
- Facility and equipment are in place.
- Routine Production Started
- 2013: Production of 35 t targeted
- 2014: Capacity of 50-60 t

Artemisinic Acid → Light → Artemisinin

SANOFI

OneWorld Health, a drug development affiliate of PATH
Conclusion

- Shows that innovative technologies (like synthetic biology) can be implemented industrially rather quickly and can thus promptly contribute to global health.
  - From Lab realization to Industrial scale incl. regulatory approval in about 10 years. Industrialization phase: about 4 years
    - Rather complex process – fermentation step & chemical steps
- Partner with large network of industrial technologies/facilities and expertise is at least helpful if not necessary to realize such a project.
  - Sanofi group: Overall 112 industrial sites in 41 countries
    - 16 sites in Chemistry & Biotechnologies (active ingredient)
    - 10 sites in vaccine production (Pasteur)
    - More than 40,000 employees in Production
Conclusion

• Success story of cross-sector partnership among industry, academia and nonprofit organization.
• New commercial-scale alternative manufacturing process to produce a complementary source of artemisinin and supplement the plant-derived supply.
• Pivotal milestone in the fight against malaria.
Thank You

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Photo: PATH/Laura Newman