

# Technology background



## Cilian at a glance

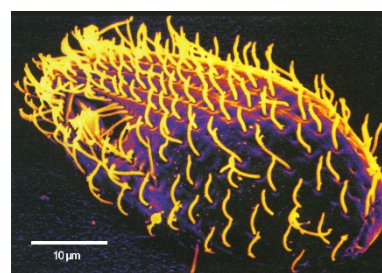
Cilian is an innovative biotechnology company, with 900 m<sup>2</sup> laboratory space, a 900 L pilot scale bioreactor and 30 employees. It uses the singular abilities of Ciliates, a class of eukaryotic micro-organisms, for the industrial production of therapeutic proteins. The company's R&D team has more than 20 years of experience in the applied research and development of its expression system based on Ciliates. Since Marcus Hartmann, founder of Cilian AG, formed the R&D team in 2001, Cilian has developed and achieved a patented, marketable expression technology, brand named CIBEX

system that has been optimized for the industrial production of biopharmaceuticals. Worldwide, Cilian is the only biotech company to apply a system such as CIBEX with corporate partners. CIBEX is marketed by the a management team consisting of Christian Scheiner (CEO), Marcus Hartmann (CSO) and Christian Fründ (CFO) and is ready to be utilized for

production of a variety of biopharmaceutical compounds, specifically therapeutic enzymes, monoclonal antibodies with improved effector function as well as viral and protozoan vaccines. Therapeutic targets are the treatment of digestive disorders, cancer accessible to monoclonal antibody immunotherapy and viral infectious diseases, just to name a few.



Christian Scheiner (CEO) Marcus Hartmann (CSO) Christian Fründ (CFO)



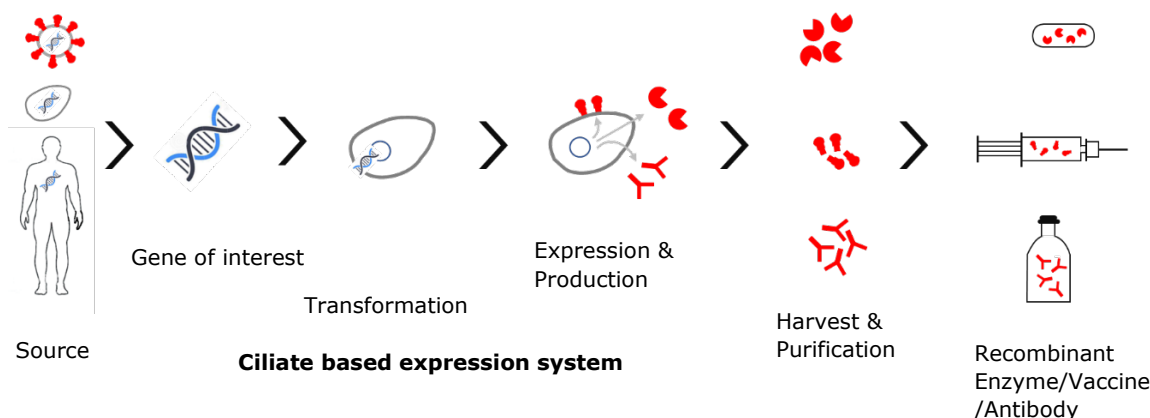
Raster electron microscopic image of *Tetrahymena thermophila*

## Expression and production technology

The Ciliate based expression system (CIBEX) is efficient, safe and cost effective in the production of biopharmaceuticals. Ciliates of the species *Tetrahymena thermophila* are eukaryotic single cell micro-organisms, which are characterized by unique cell biology and genetics. High AT-content, the non-canonical codon usage and missing evidence of viruses from higher animals have shown that viruses cannot use Ciliates as hosts for proliferation. Thus, the cell biology and the genetics of

Ciliates make this micro-organism ideally suited for the safe production of biopharmaceuticals. CIBEX already reached market standard parameters for yield and costs of goods. With CIBEX, the most modern expression system for the pharma industry is now available, which has proven to be superior to currently used expression systems and is not subject to limitations of some of those systems. The applicability and transferability of CIBEX to

proteins with different characteristics has already been established in feasibility studies. To date, Cilian has demonstrated the successful expression of 18 biopharmaceutical proteins with a space time yield between 50 to 1000 mg per liter per day. CIBEX has been applied to three in-house product developments: a recombinant flu vaccine, an anti-tumor antibody and an enzyme preparation. All three successfully passed proof-of-principle stage.



## Characteristics of the Biotechnology of Ciliates

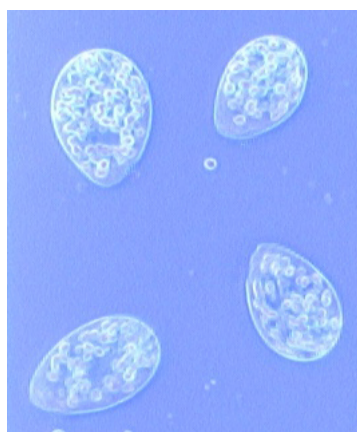


- batch, fed batch and continuous cultivation in existing microbial bioreactor equipment possible
- Advantageous and consistent oligo-mannose N-glycosylation (no micro-heterogeneity);
- no hyper-glycosylation; no adverse residues, induction of ADCC effector function by MAbs
- Ease of scale up - proven in 7.500 liter stir tank and 1.500 liter air lift batch fermentation
- *In vivo* efficacy of products in pipeline proven



7,500 liter fermentation of *Tetrahymena* at CDMO side

## Benchmark parameters of a typical fermentation process



Light microscopic image of *Tetrahymena thermophila*

Parameter	Value
Generation time	4 - 6 h
Estimated costs medium	2-8 Euro/liter
Stirrer tip speed	2-3 m s <sup>-1</sup>
Volumetric productivity (space time yield)	50 - 1,000 mg/liter/day - extended fed batch (for selected biopharmaceutical proteins)

## Results today

- Expression of 18 biopharmaceutical proteins.
- Proof of principle studies completed for:
  - a recombinant quadrivalent flu vaccine (CiFlu)
  - an anti-tumor antibody rituximab (CiMab)
  - an enzyme preparation against exocrine pancreatic insufficiency (Cilase)

## Contact

[info@cilian.de](mailto:info@cilian.de)

+49 (251) 620 31 0



900 liter bioreactor in the labs of Cilian