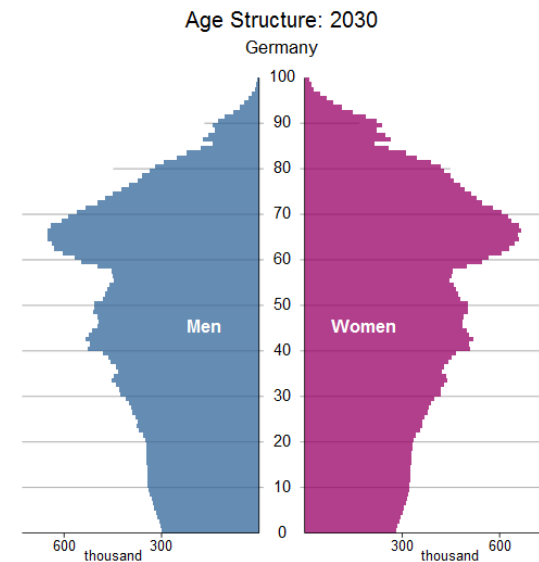
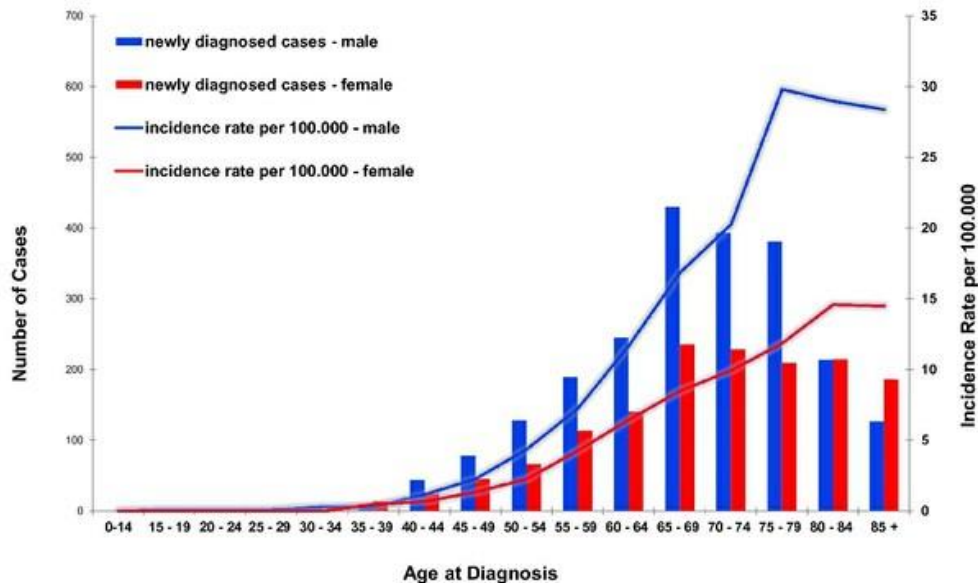


Ofatumumab in CLL:  
Evaluation of in-vitro response  
mechanisms

Matthias Volden

# About CLL

- CD19+, CD5+, CD20+, > 5.000 cells/ $\mu$ l blood
- bone marrow, lymph nodes, liver, spleen
- heterogenous clinical course: risk stratification



<https://www.destatis.de/bevoelkerungspyramide/>

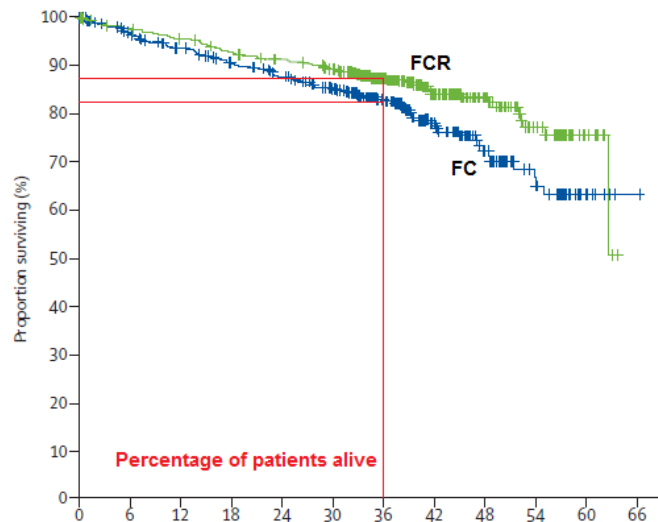
(Source: Ulrich Gerdemann, Ron Pritzkeleit, Alexander Katalinic, Cancer Epidemiology Institute, Lübeck, Germany)

Projection of the Cancer Epidemiology Institute, Lübeck, for ICD10: C91.1 based on the number of new cases derived from the Cancer Registers of Bremen, Hamburg, Lower-Saxony, Münster, Saarland (for ICD 9: 204.1) and Schleswig-Holstein in the years 2007/8 (reference population  $\approx$  16 million inhabitants), September 2011

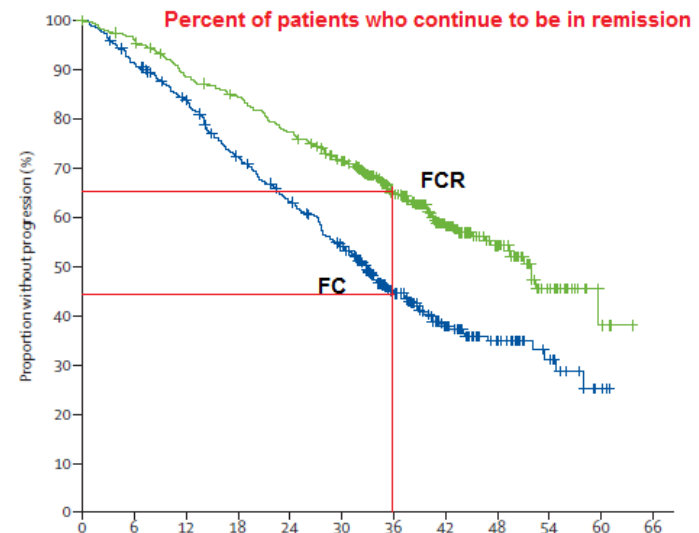
# Therapy of CLL

- Chemotherapy (Fludarabine, Bendamustine)
- CD20 antibodies (Type I: Rituximab, Ofatumumab, Type II: Obinutuzumab)
- New substances (Ibrutinib)

overall survival

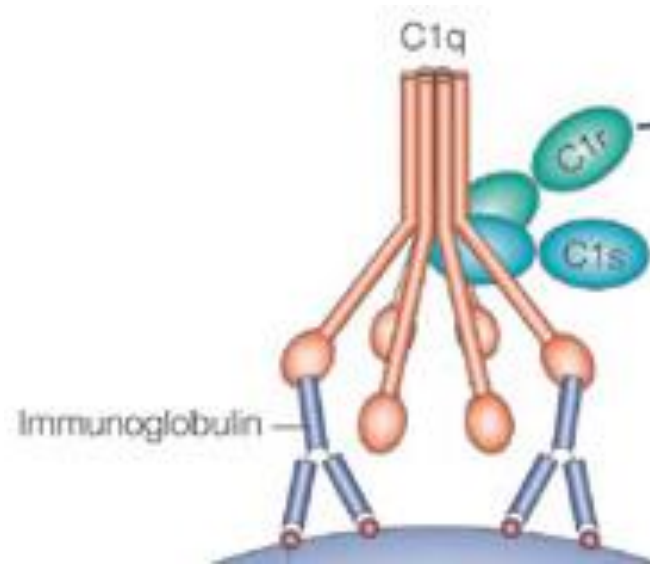


progression free survival



# Mechanisms of antibodies

- ADCC
- CDC
- PCD
- Growth inhibition
- Chemo-sensitization
- Blocking of e.g. survival stimuli
- Atypical, new mechanisms: homotypic adhesion, cross-linking-related...



Timothy J. Foster [Immune evasion by staphylococci](#)

Nature Reviews Microbiology 3, 948-958 (December 2005)

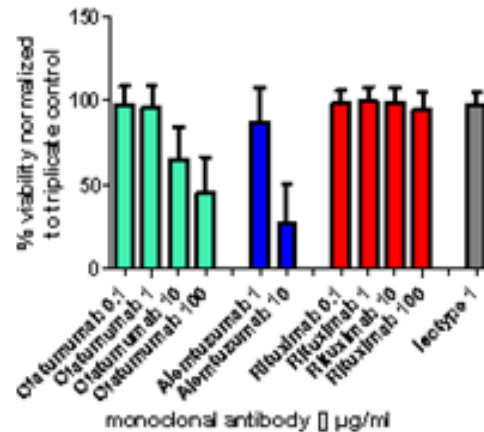
# Methods

- Patient characterization:
  - clinical data: age, leukocyte count, pre-treatment
  - genetic classification, risk stratification (refractory, 17p del, *TP53* mutation)
- Cell culture under different conditions:
  - primary CLL cells and fresh-frozen serum
  - Co-culture with nurse-like cells (HS5 cells)
  - whole blood culture (fresh CLL patient blood)
- Read-out after different incubation times:
  - Microscopy (to investigate homotypic adhesion)
  - luminescence-based cell viability assay
  - Flow Cytometry (True Count, Annexin-V/7-AAD-staining)

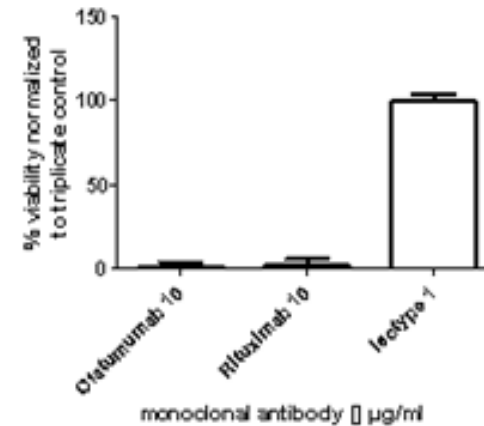
# Results (I) (3 hours; 48 hours)

## A) CLL-cells

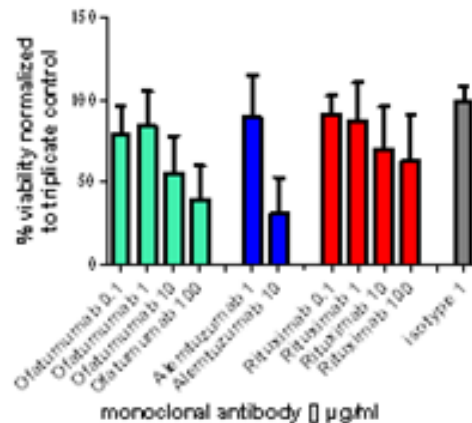
3h incubation, 37°C, 5% CO<sub>2</sub>, 1\*10<sup>4</sup> cells



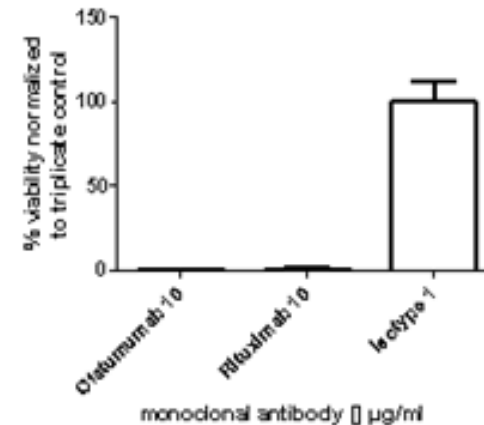
## B) Raji-cells



## C) 48h incubation, 37°C, 5% CO<sub>2</sub>, 1\*10<sup>4</sup> cells

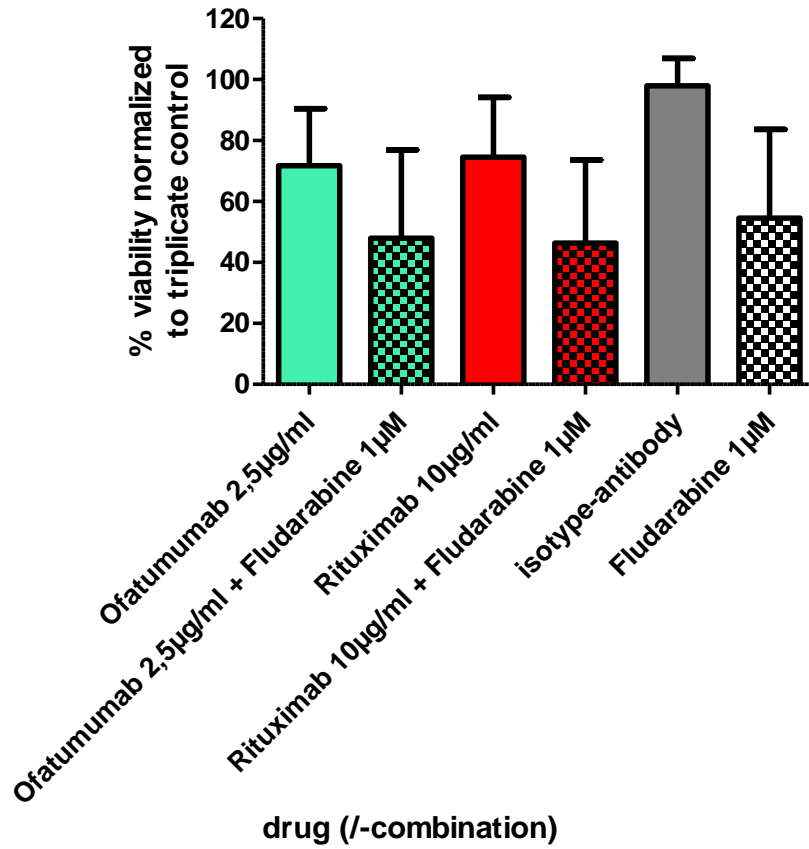


## D)

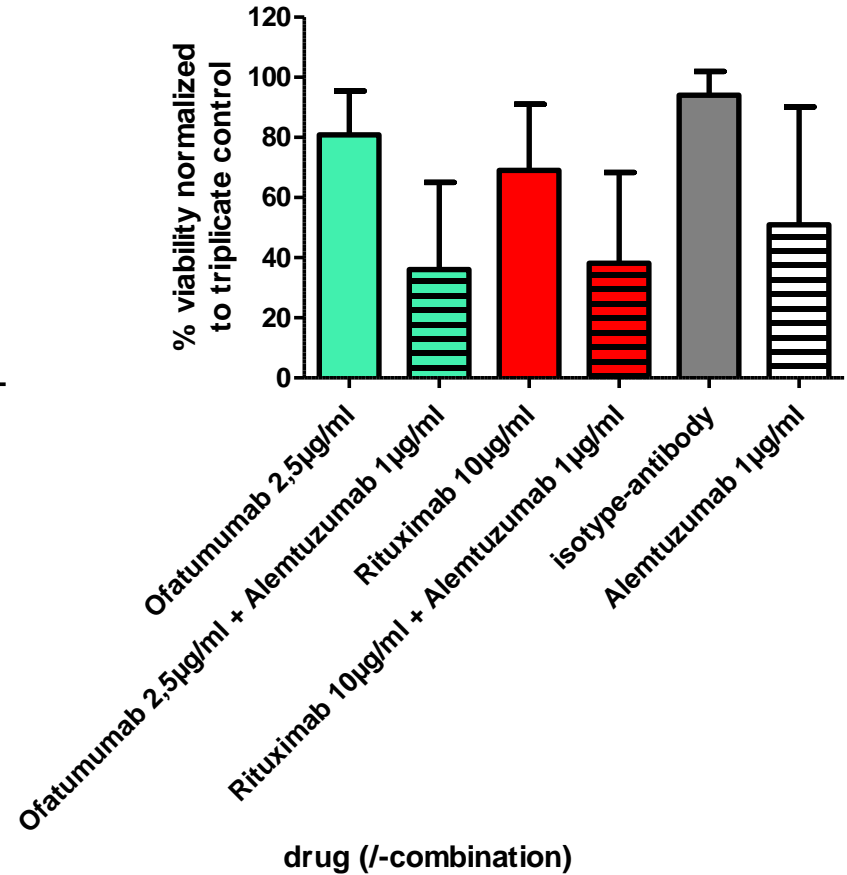


# Results (II) (48 hours)

combination with Fludarabine, n=25

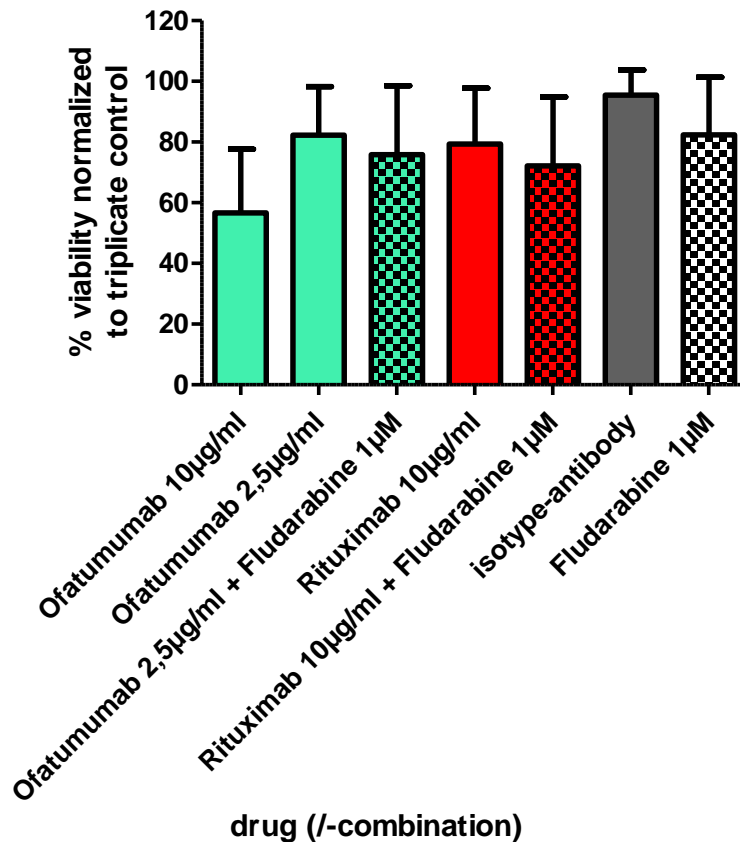


combination with Alemtuzumab, n=12

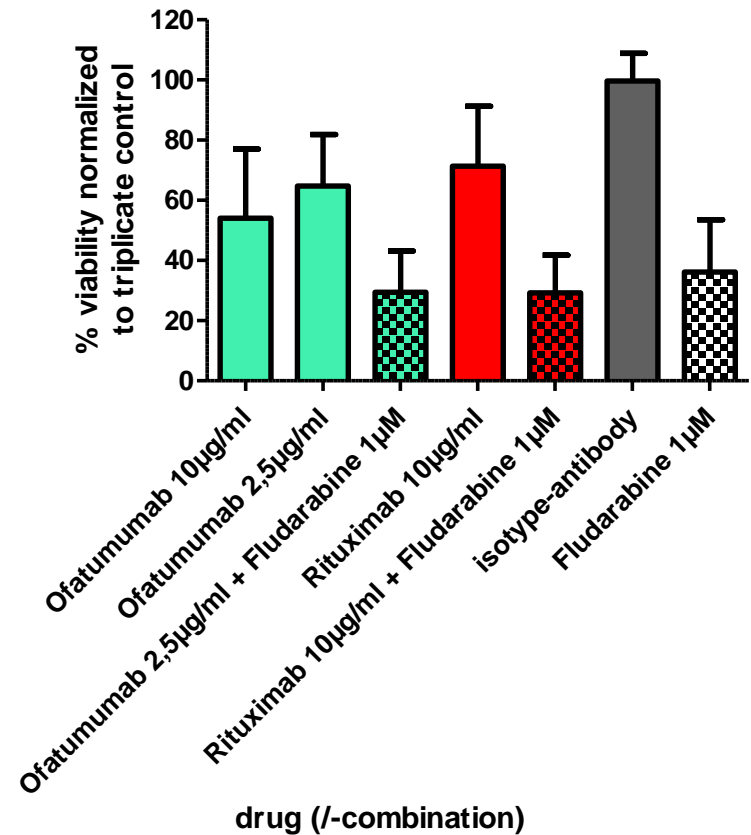


# Results (III) (48 hours)

combination with Fludarabine high risk, n=10



combination with Fludarabine low risk, n=15





# Summary

- High risk vs. Low risk
- Pretreated vs Untreated
- Complement required (CDC, complement dependent intra-cellular effects?)
- No homotypic adhesion
- Different antibodies, different mechanisms
- Combination possibilities
  - combination with chemotherapy
  - combination of antibodies
  - further options

# Outlook

- Comparison of clinical outcome and preclinical data
  - pretherapeutic testing of patients?
  - individual adjustment of therapeutic approach
- Clinical trials for new combinations?
- Identification of natural cross-linker (C1q?)
  - addition of cross-linker as therapeutic option?
- Focus on resistance mechanisms (Notch-1?)

“targeted use of targeted therapy“