

# Preclinical evaluation of a type II CD20 monoclonal antibody (GA101) in CLL

Alex Fisher

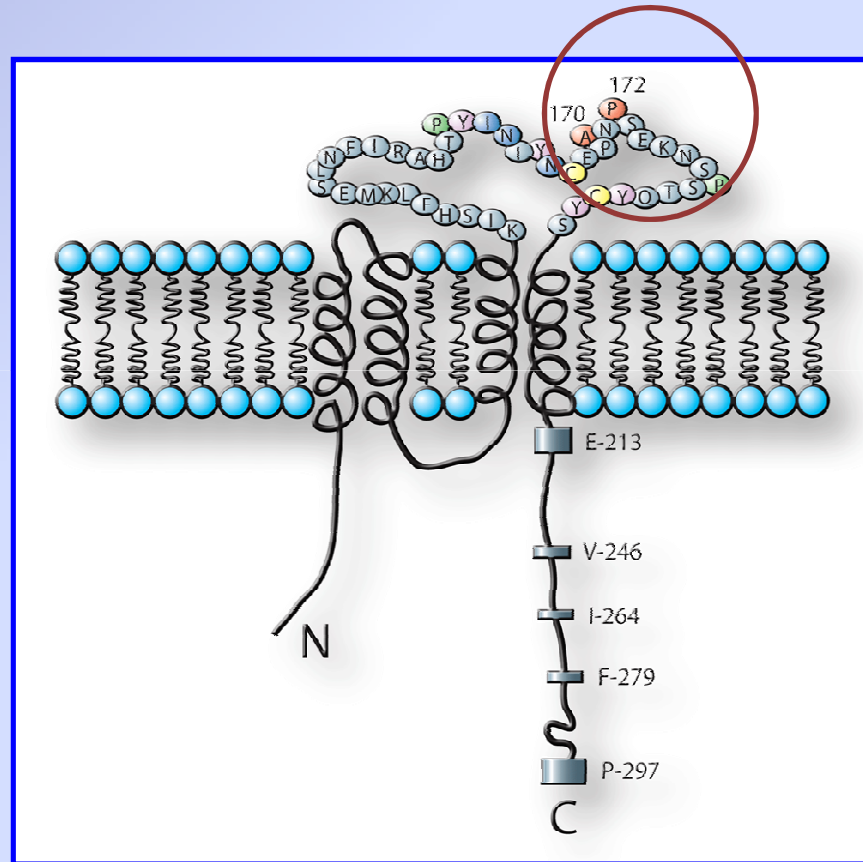
MRC Toxicology

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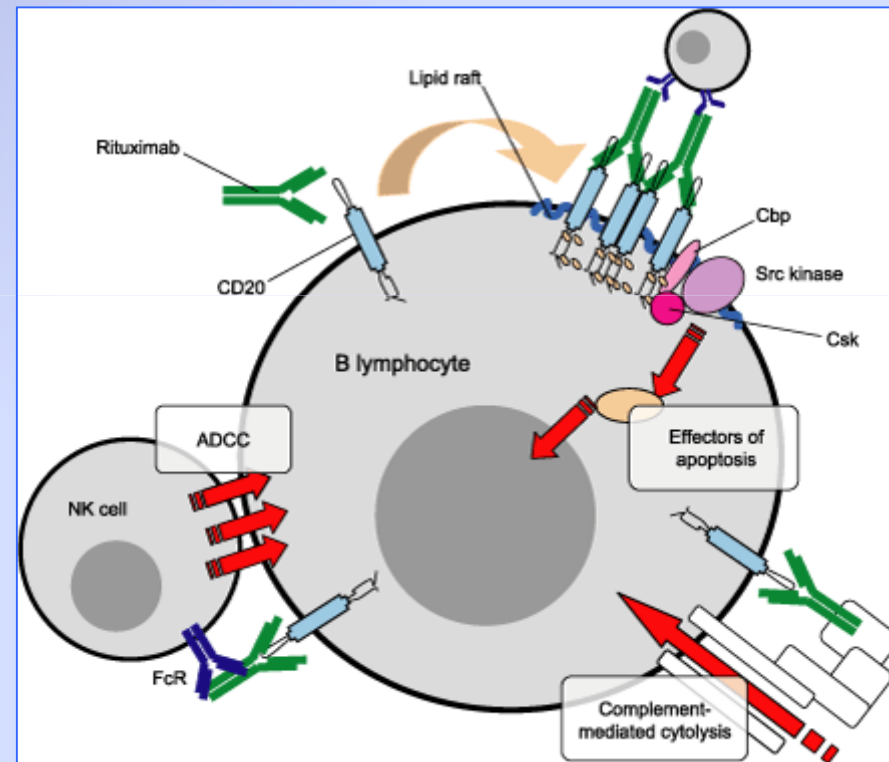
# Anti-CD20 Monoclonal Antibodies (mAb)

- Rituximab first monoclonal antibody approved to treat cancer.
- Combines synergistically with existing chemotherapeutic regimens and has significantly improved survival.
- Does not exhibit long-term toxicity and short term toxicity is not synergistic with chemotherapy.



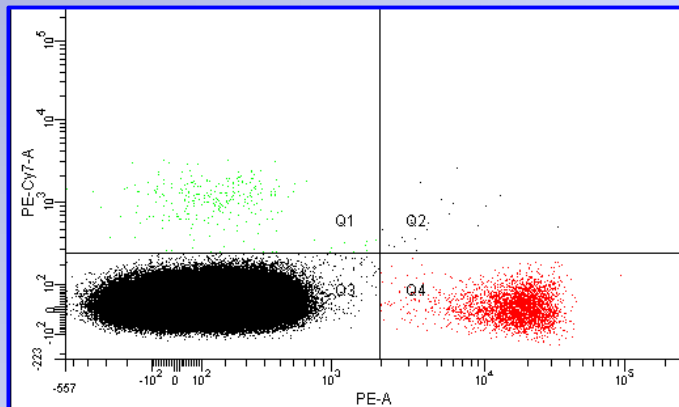
# Why is CD20 such a good target?

- Targeting of CD20 is thought to elicit antibody dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC) and programmed cell death (PCD)

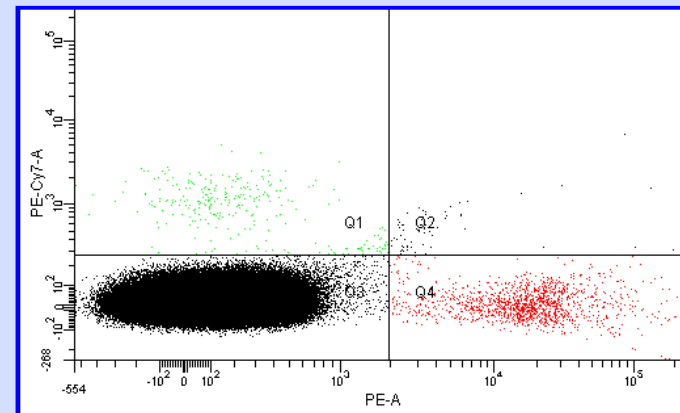


# *In vitro* Whole Blood Assay

- Whole blood from patients with a range of B cell malignancies was treated with GA101 (10 $\mu$ g/ml) and Rituximab (10 $\mu$ g/ml).
- At 4 and 24 h time points **CD3** positive cells and **CD19** positive cell ratio assessed using flow cytometry.



Untreated



GA101 treated

# Results of Whole Blood Assay

- 85 different patients - 45 of these patients CD20 expression quantified.
- GA101 consistently results in greater depletion of B cells compared to Rituximab (strong correlation with CD20 antigen expression).
- No correlation between prognostic markers including: interphase FISH abnormalities (11q), *IGHV* mutation and CD38 expression.
- GA101 mediated depletion of B cells observed in lymph node and tonsil tissue.

# Results of whole blood assay

The percentage depletion of B cells in response to treatment with GA101 or Rituximab.

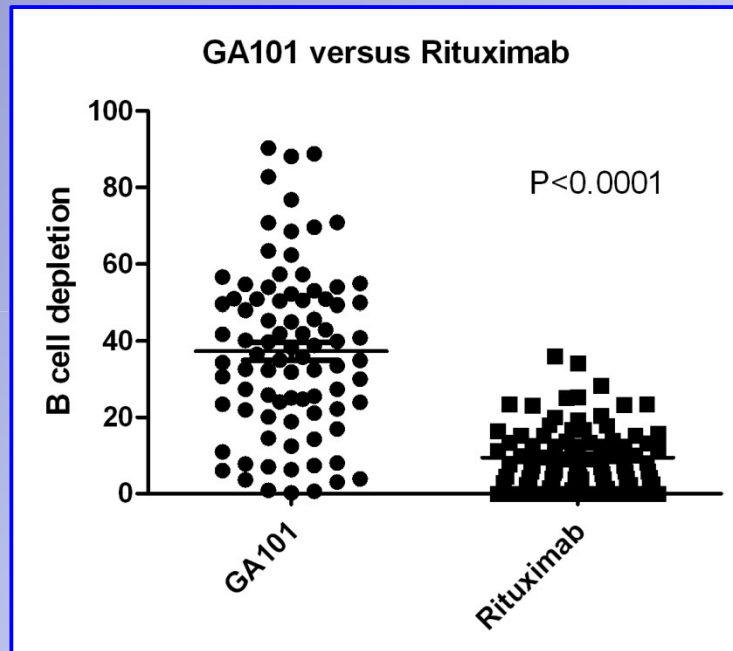
	<b>GA101</b>	<b>Rituximab</b>
<b>MEAN</b>	37.4	9.5
<b>MEDIAN</b>	36.5	7.3
<b>RANGE</b>	0 - 90	0 - 36
<b>N</b>	85	85

85 patients were analysed in total.

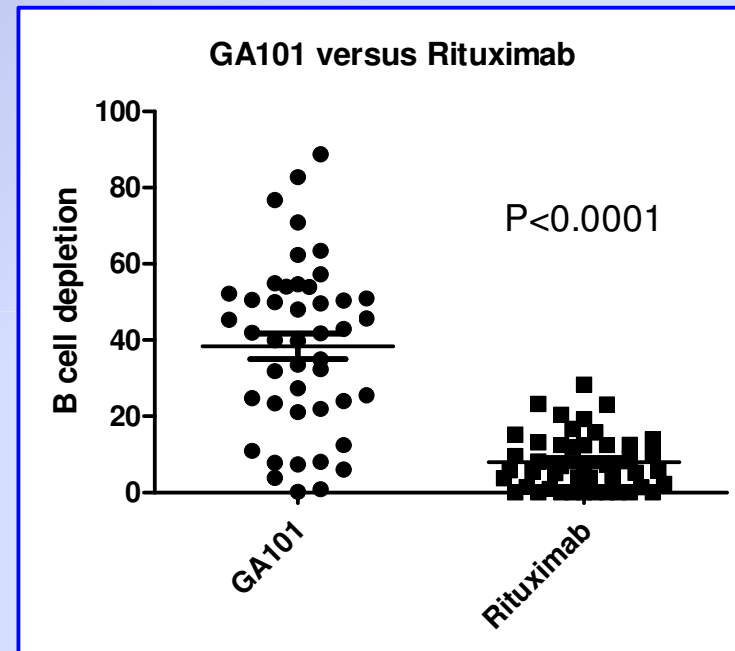
	<b>GA101</b>	<b>Rituximab</b>	<b>CD20 expression</b>
<b>MEAN</b>	38.4	7.9	129023
<b>MEDIAN</b>	41.8	5.9	58194
<b>RANGE</b>	0 - 90	0 - 36	10924 - 1224437
<b>N</b>	45	45	45

CD20 surface expression was quantified in 45 patients

# GA101 displays superior B cell depletion compared to Rituximab

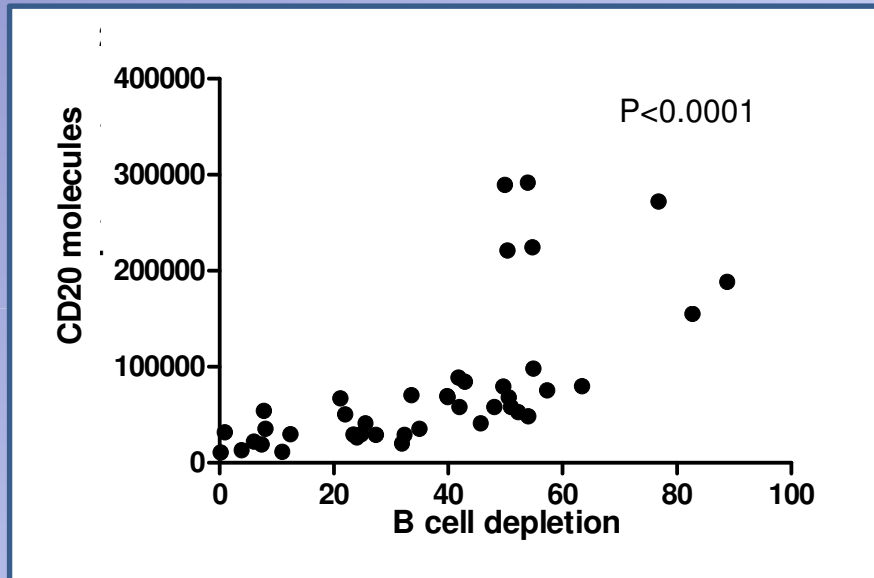


All B cell malignancies

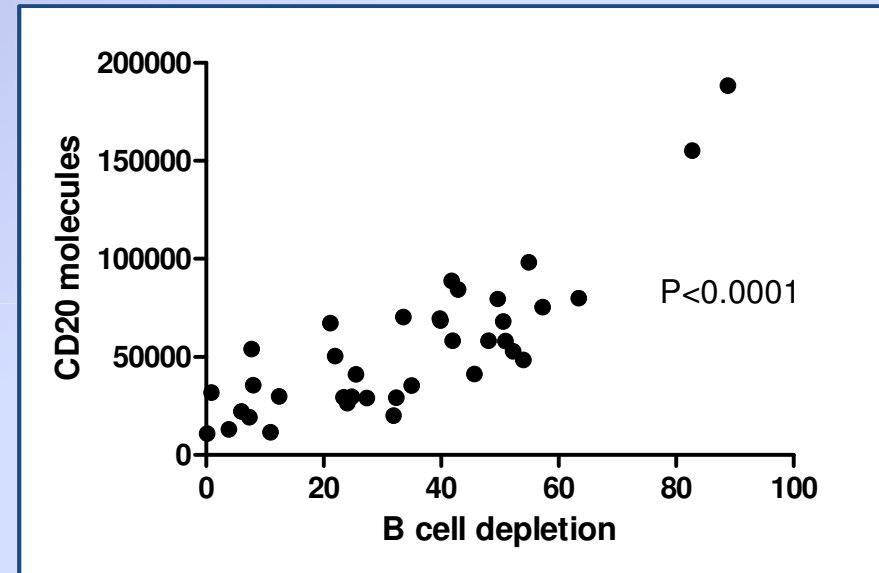


CLL

# CD20 expression correlates with B cell depletion



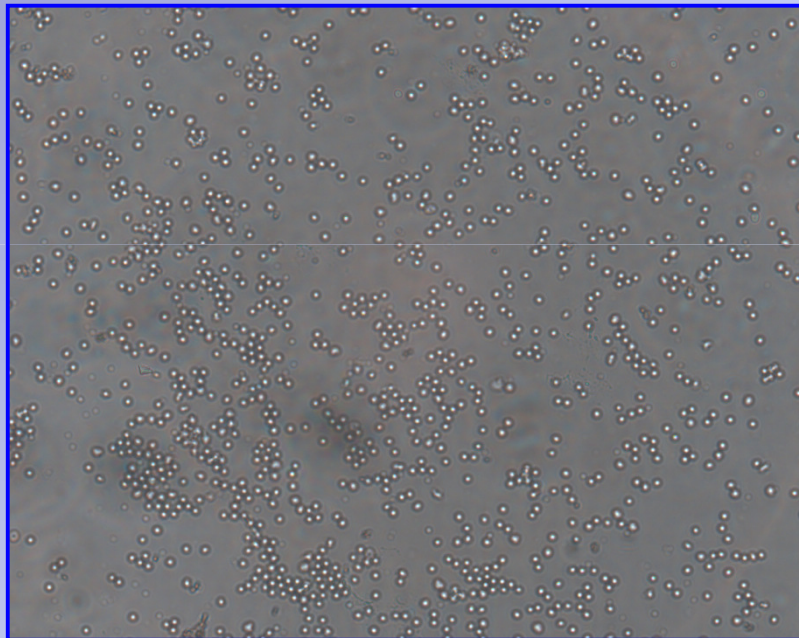
All B cell malignancies



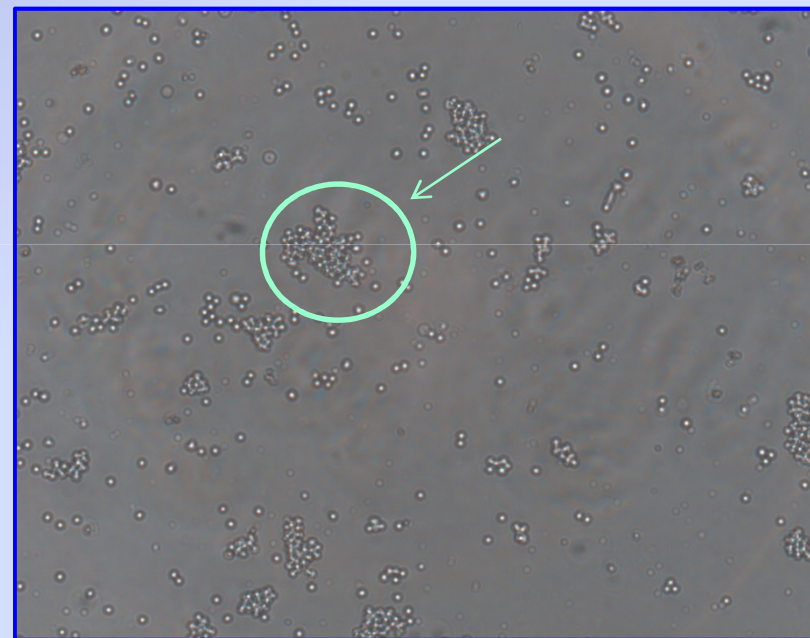
CLL

# Homotypic adhesion (HA) in ficoled, CLL cells

Control T = 24

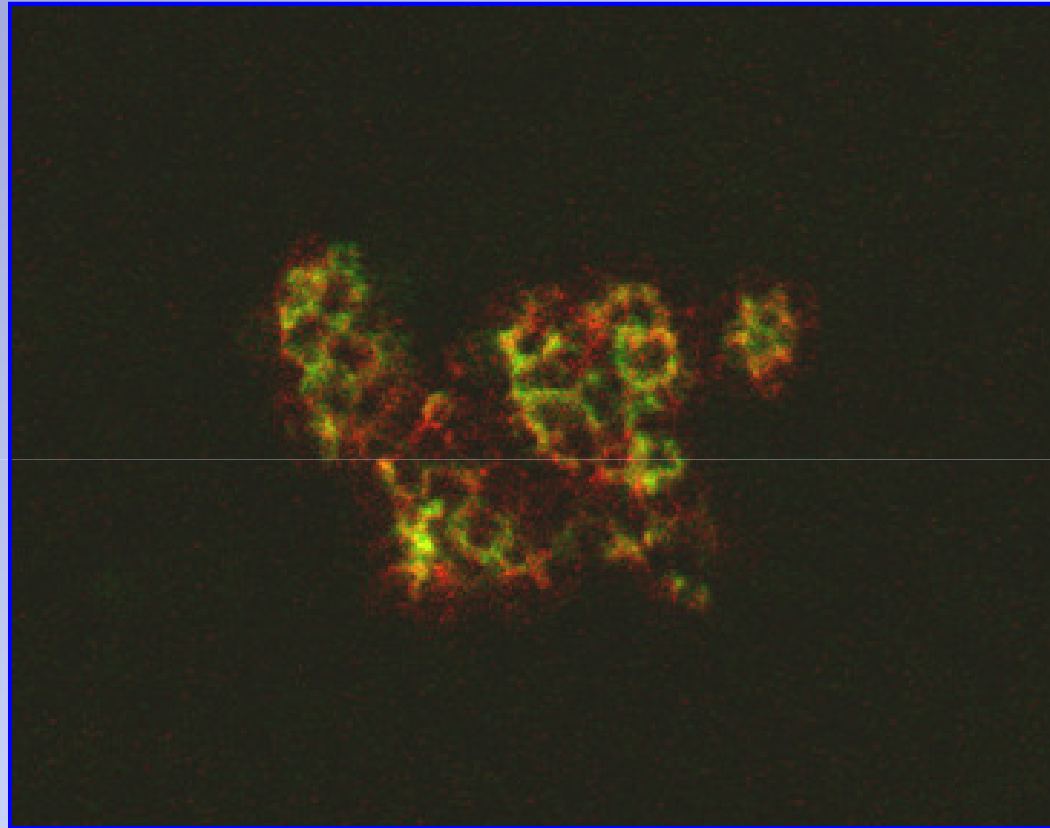


GA101 treated T = 24



Homotypic adhesion is at least partially responsible for the observed B cell depletion in the whole blood assay.

# HA in whole blood



Whole blood from patient with CLL stained treated with GA101 (10 $\mu$ g/ml ) for 24 hours and then stained with **CD19-PE** and **Annexin-FITC**

# Ongoing Work

- Characterising the details of HA.
- Looking for synergy with GA101 and other agents.
- Investigating various means of up-regulating CD20 expression.

# Acknowledgements

Prof. Martin Dyer  
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Dr. Ben Kennedy  
Dr. Fiona Miall

Lab 402  
Roche

And patients!



# GA101

- Next generation antibodies against CD20 have been developed, which are resistant to internalization and have improved function.
- GA101 is a third generation type II IgG1  $\kappa$  anti CD20 mAb.
- Humanized mAb made by grafting the complimentary determining regions of murine mAb B-ly1 to human IgG1  $\kappa$  sequences
- Putatively, has enhanced ADCC due to non-fucosylated Fc region in addition to improved PCD capabilities.