Quantification of Bispecific Antibody Mediated T-cell Activation

Engineered CD3 Effector & tailored CD19+ Target Cells

Segerstein T, Frier Bovin L, Rodó J, Gobbo E, Ferrando-Miguel R, Schwenkert M, Lallemand C
Svar Life Science AB, Malmö, Sweden

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INTRODUCTION

Bispecific antibodies, with their numerous formats, are advancing cancer immunotherapy, particularly in hematologic malignancies. Created from components like immunoglobulin half molecules, Fab fragments, or single chain Fvs, they can recruit and activate immune cells or interfere with receptor signaling. Over 100 such antibodies are in clinical development, demonstrating various modes of action.

Blinatumomab, a Bispecific T-cell Engager (BiTE) molecule, targets CD3 and CD19 for T-cell mediated elimination of B-cell malignancies. CD19 is an attractive target because early B-cell malignancies arise from CD20-negative Pro-B and Pre-B cells, unlike Rituximab, which targets CD20. Consequently, blinatumomab was approved for aggressive B-cell precursor acute lymphoblastic leukemias (ALLs), often affecting children.

This success has spurred development of other bispecific molecules for hematologic malignancies, with over two-thirds targeting CD19 and CD20 and 90% targeting CD3 on the effector side. Current analytical methods for T-cell activation have limitations, but our improved bioassay platform using CD3xCD19 offers a more reliable assessment. This approach involves effector T-cells carrying a reporter gene downstream of the CD3 signaling cascade and engineered target cells as antigen-positive/-negative controls.

In this study, we hijack a signaling pathway to engineer specific expression of Firefly Luciferase, with a second Luciferase for normalization. Effector human T-cells are engineered to transduce CD3 activation signal into Firefly Luciferase expression, while Renilla Luciferase is controlled by a household gene. We also use engineered human CD19-positive B-cells for target-specific response.

This pair of CD19 +/- target cells is ideal for assessing antigen-specific T-cell activation. All these cells are provided in a thaw-and-use assay format, decreasing timelines while offering reliable results.

Both the *iLite* CD3 Effector Assay Ready Cells and the *iLite* CD19 (+) Target Assay Ready Cells, were used to analyze signal induction by blinatumomab.

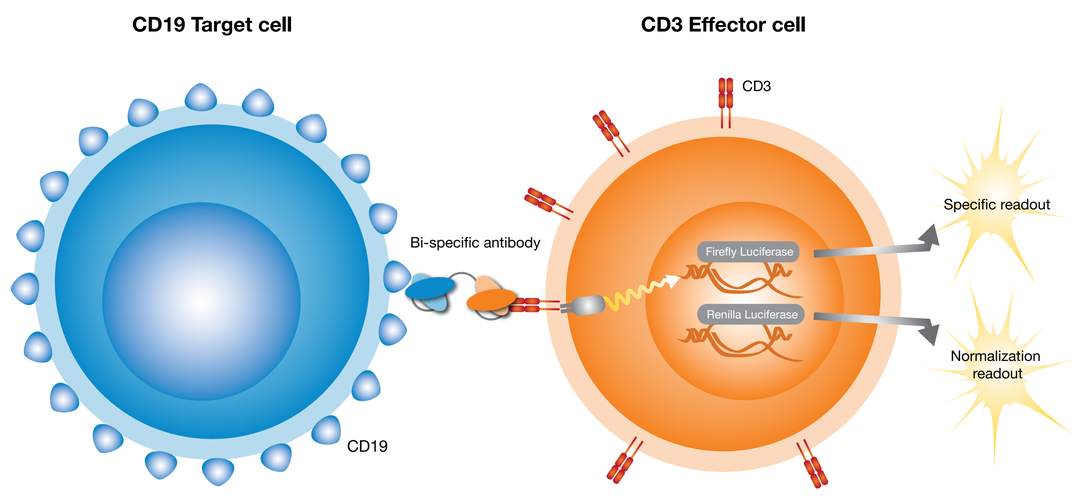


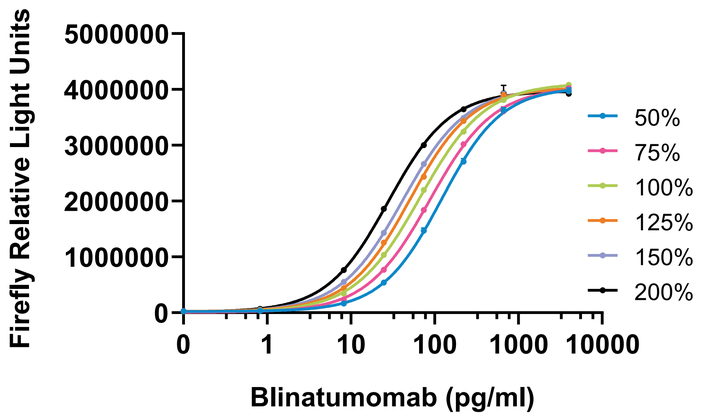
Fig 1. *iLite cell-based technology principle*

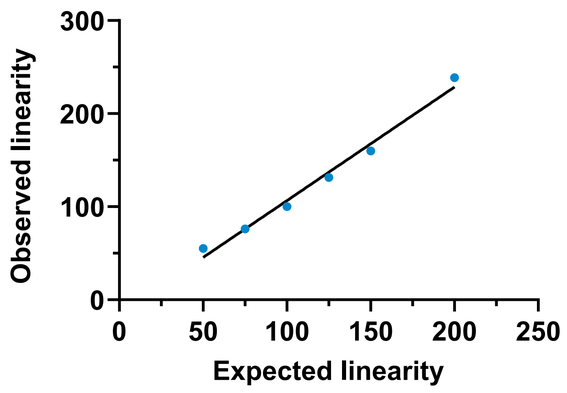
POTENCY

We conducted a linearity assay with varying concentrations of Blinatumomab to demonstrate the correlation between their concentrations and the level of induced firefly expression.

The obtained results were plotted against a 100% reference curve, enabling us to determine the linearity between the expected EC50/IC50 and the measured EC50/IC50 values.

Expected linearity	50%	75%	100%	125%	150%	200%
EC50	121	88	67	51	42	28
FL induction	91%	99%	na	95%	94%	84%
Hill Slope	1.2	1.1	1.1	1.2	1.2	1.2
Fold Induction	132	143	152	145	154	148





Potency assessment:

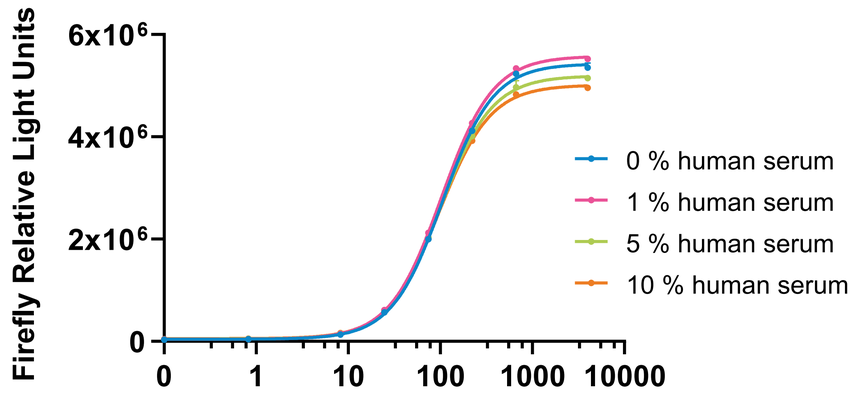
The iLite CD3-CD19 bioassay was performed with one reference dose-response curve, called 100% and with concentrate at 50, 75, 125, 150 and 200% of the reference.

SERUM TOLERANCE

In order to determine the impact of serum matrix on the assay outcomes in clinical samples, we performed experiments using different serum concentrations.

This enabled us to identify the serum's effect on the assays and ascertain the maximum serum concentration (tolerance) for the assay.

Serum concentration	0%	1%	5%	10%
Hillslope	1.6	1.5	1.5	1.6
EC50	106	103	100	97
Fold induction	153	150	127	114



Serum effect/tolerance:

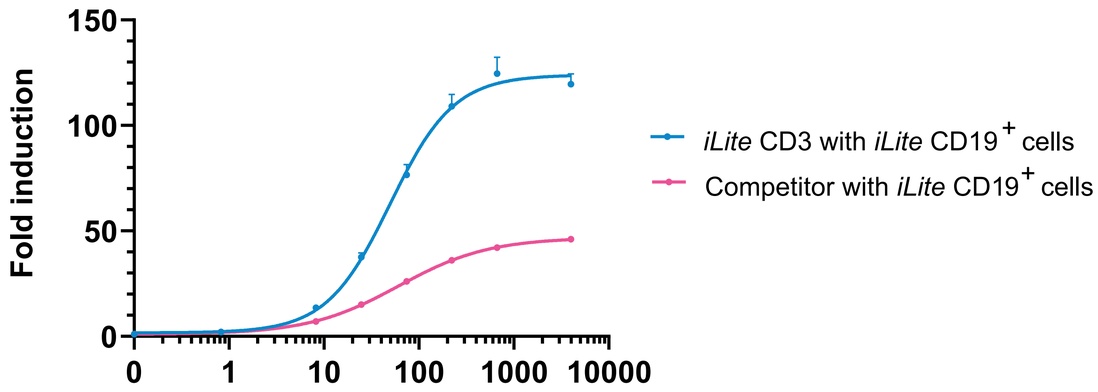
The iLite CD3-CD19 bioassay was performed with a dose-response curve including human serum, up to 10%. As seen in the figure, the presence of serum is well tolerated in the assay.

COMPETITOR COMPARISON

The efficacy of *iLite* CD3 Effector Assay Ready Cells was evaluated against an alternative effector cell line available in the market. In both assays, *iLite* CD19 (+) Target Assay Ready Cells were utilized to analyze the signal induction by blinatumomab.

The results revealed a remarkable 130-fold induction of the RLU signal for *iLite* CD3, outperforming other available effector cells in the market.

	iLite CD3 - CD19	Competitor with *iLite* CD19 cells
EC50	49.78	61.15
Fold induction	130	45



Comparison:

The iLite CD3 effectors cells were compared with the market-leading alternative using the CD19+ target cells.

CONCLUSION

The use of cell-based assays for detecting and measuring T-cell activation presents an opportunity to create more advanced bispecific therapeutical antibodies. With this goal in mind, we have generated a new platform for evaluating the activation of T-cells, showcasing it´s possibilities using Blinatumomab, a bispecific CD3-CD19 antibodies. It is composed of the *iLite* CD3 effector cells and engineered *iLite* CD19+ target cells.

The platform performance results indicate that the *iLite* platform has a low intra-day and intra-lot variance (data not shown) as well as it shows the good correlation between the concentrations and the level of induced firefly expression in a serials of dilutions.

Furthermore, the presence of serum is well tolerated in the assay, as demonstrated in the Serum effect/tolerance experiment. Finally, the data also shows that the *iLite* CD3 Effector Assay Ready Cells outperformed other available effector cells in the market, with a remarkable 130-fold induction of the RLU signal.

- **Potency assessment demonstrated the correlation between varying concentrations of Blinatumomab and the level of induced firefly expression.**
- **Both cell lines present a great serum tolerance with an almost non-existent shift on EC50 or hill slope values.**
- **The *iLite* CD3 Effector Assay Ready Cells outperformed other available effector cells in the market, with a remarkable 130-fold induction of the RLU signal.**

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