Efficacy of Cavatak against CD40L-stimulated CLL

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Chronic lymphocytic leukaemia (CLL)

- B-cell malignancy that most commonly affects older patients
- Often runs an initially indolent course but requires treatment once symptoms develop
- Induction chemotherapy generally results in excellent responses but the disease is rarely eradicated and invariably recurs, eventually becoming refractory to treatment
- Treatments are needed that could purge minimal residual disease with minimal associated toxicity

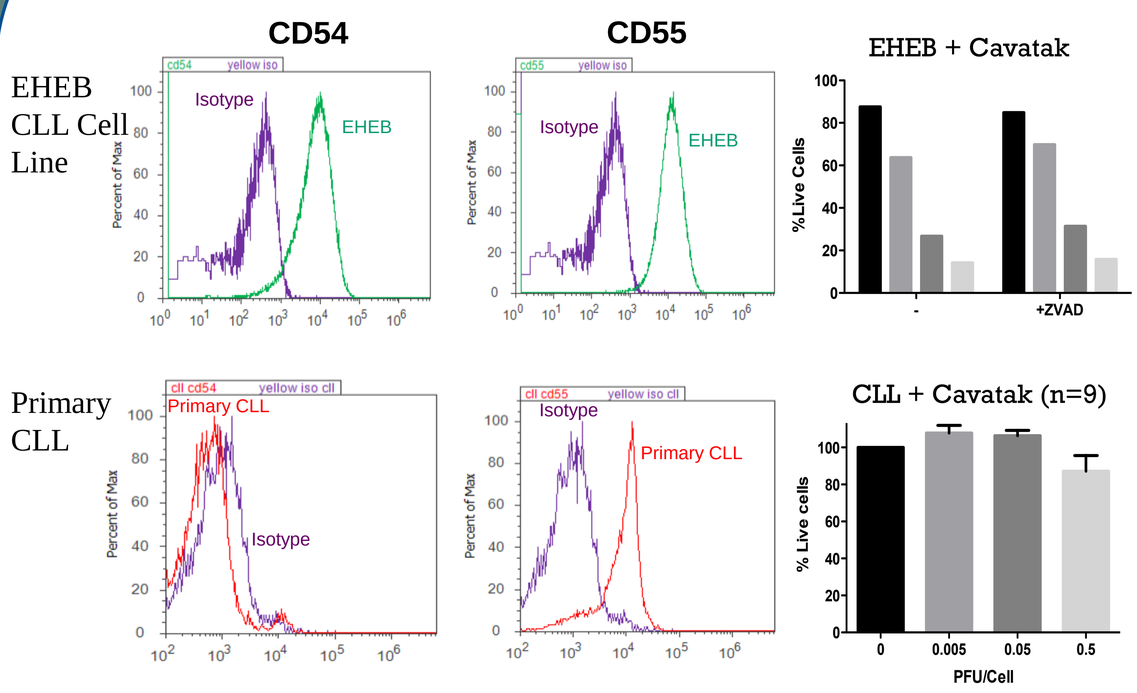
Cavatak

- Unmodified coxsackievirus (A21) oncolytic virus
- Preferentially infects cells that overexpress CD54 (ICAM-1) and its co-receptor CD55 (Decay accelerating factor)
- Well tolerated in a clinical trial of intratumoural administration for melanoma
- Phase I trial of intravenous delivery for solid tumours has now opened

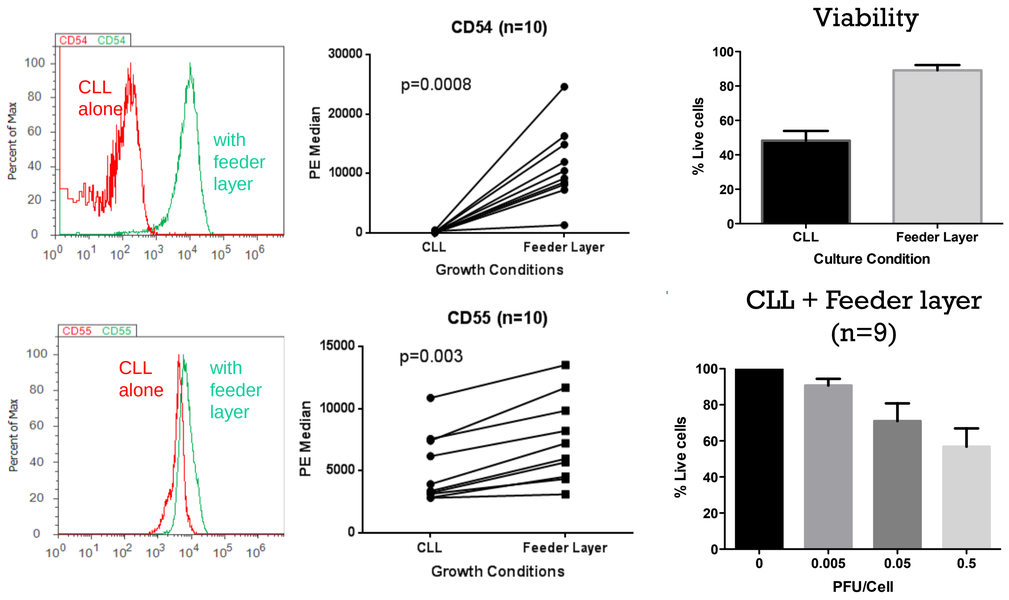
Methods: This study used a combination of established human CLL cell lines (EHEB, MEC2) and primary CLL samples from patients with high malignant cell counts (n=9). CLL cells were isolated from primary samples using lymphoprep; the isolated PBMCs contained over 90% CD19+/CD5+ malignant CLL cells on flow cytometry. Cytotoxicity was quantified using the Live/Dead assay.

In order to model the situation of resistant disease residing in lymph nodes, a potential source of minimal residual disease, we co-cultured CLL cells with murine fibroblasts (L929), transfected to express CD40L, for 48 hours prior to virotherapy.

Cytotoxic effects of Cavatak



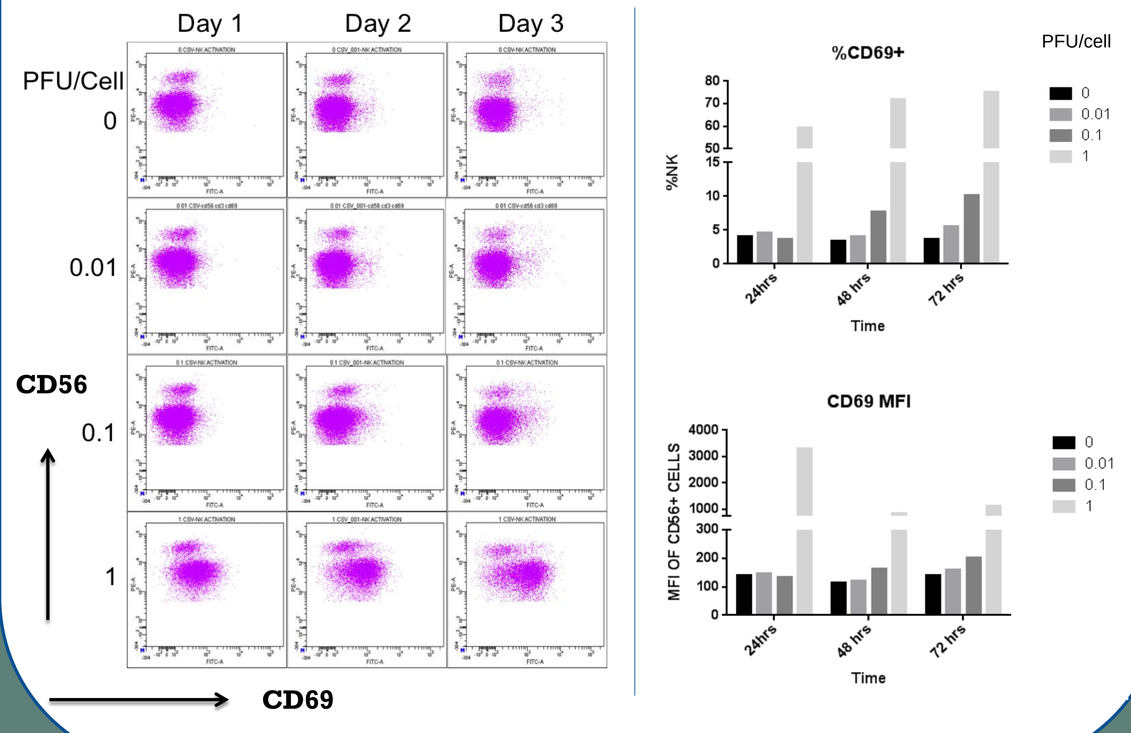
Primary CLL samples express low levels of CD54 and are resistant to Cavatak in contrast to the CLL cell line EHEB which expresses high levels of CD54 and is susceptible to Cavatak. Both cell types express the co-receptor CD55. Inhibition of caspases (by the inclusion of ZVAD-fmk) had no affect on cell death induced by Cavatak in CLL cell lines.



CD40L stimulation was used to mimic lymph node resident disease. *Ex vivo* CLL samples were stimulated for 48 hours before analysis by flow cytometry. Viability was increased compared to cultures with no stimulation as was CD54 and CD55 expression. CD40Ligand stimulation also resulted in an increased sensitivity to Cavatak treatment.

Innate immune effects of Cavatak

PBMC from healthy donors were incubated with Cavatak and CD69 expression examined on CD56+CD3- cells:



Results: Cavatak demonstrated cytotoxic effects when administered to CLL cell lines but no effect against primary samples in monoculture. Interestingly, when primary CLL cells were incubated with CD40L-expressing mouse fibroblasts increased expression of both CD54 and CD55 was observed.

The efficacy of Cavatak virotherapy was increased after co-culture with CD40L positive cells. CLL cell lines are susceptible to Cavatak virotherapy but human primary cells were resistant. In our model of lymph node resistant disease, primary CLL cells upregulate their expression of CD54 and CD55, in response to CD40L expression on neighbouring cells, thus mimicking T cell/CLL interactions that occur within the lymph node. In turn primary CLL cells become susceptible to treatment with Cavatak, as demonstrated by Live/Dead flow cytometry.

Initial data suggest that Cavatak may enhance the cytotoxic activity of NK cells, which has been shown to be an important element of virotherapy with other agents.

This suggests that Cavatak could have activity for CLL cancer patients and further trials should be considered for therapy of MRD in CLL patients.

Conclusions:

- Expression of the Cavatak receptor CD54 (induced by CD40Ligand stimulation) on primary CLL samples increases their susceptibility to Cavatak.
- Cavatak may have efficacy in CLL patients with minimal residual disease in lymph nodes.
- Cavatak can activate healthy NK cells in a dose and time dependent manner and thus could potentially induce an anti-tumour response if this is also seen in cancer patients.

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