

Unexpected mechanisms of action for a cytokine receptor-blocking antibody

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Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; AML, acute myeloid leukemia; ALL, B-cell acute lymphoblastic leukemia; CART, chimeric antigen receptor-transduced T cells; CML, chronic myeloid leukemia; GMR α , granulocyte-macrophage colony-stimulating factor receptor α subunit; IL-3, interleukin-3; IL3R α , IL-3 receptor α subunit; IL5R α , IL-5 receptor α subunit; IL13R α 1, interleukin-13 receptor α subunit 1; IL13R α 2, interleukin-13 receptor α subunit 2; LSPC, leukemic stem and progenitor cell; MDS, myelodysplastic syndromes; NK, natural killer cell; NTD, N-terminal domain; TKI, tyrosine kinase inhibitor.

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CSL362 is a humanized interleukin-3 (IL-3)-neutralizing monoclonal antibody with enhanced effector function that binds the α subunit of the IL-3 receptor (IL3R α). The crystal structure of an IL3R α :CSL362 complex shows that IL3R α adopts “open” and “closed” conformations. CSL362 blocks IL-3 function through both IL3R α conformations but via distinct and unexpected mechanisms.

The cytokine interleukin-3 (IL-3) regulates survival, proliferation, differentiation, and activation of cells of the hemopoietic and immune system (Fig. 1a). IL-3 signals through a receptor comprising the IL-3 receptor α subunit (IL3R α or CD123) and the β common (β c) subunit.^{1,2} IL3R α is overexpressed in a number of hematological malignancies including acute and chronic myeloid leukemia (AML and CML respectively), myelodysplastic syndromes (MDS), B-cell acute lymphoblastic leukemia (ALL), hairy cell leukemia, and Hodgkin's lymphoma. Compared to normal hemopoietic stem cells, IL3R α overexpression is also observed in leukemic stem and progenitor cells (LSPCs) from AML, CML, and MDS that represent a minor population of cells that give rise to the bulk tumor, but are also considered therapy-resistant and responsible for disease relapse.^{3,4} At least in the case of AML, IL3R α expression is inversely correlated with overall survival.⁵ Thus, IL3R α represents an attractive therapeutic target for a number of hematological malignancies for which a range of agents are currently being developed, including chimeric antigen

receptor-transduced T cells (CART), IL-3: diphtheria toxin conjugates, and the therapeutic monoclonal antibody CSL362.^{6,7}

CSL362 is derived from the anti-IL-3 receptor blocking monoclonal antibody 7G3,⁸ which was humanized, affinity optimized, and engineered via modifications in the Fc-domain to have a higher affinity for CD16, resulting in enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) activity (Fig. 1b and 1c).⁷ CSL362 has been shown to deplete both CML and AML blasts by autologous natural killer (NK) cells *in vitro*.^{7,9} Moreover, in mouse models of human AML and CML, CSL362 reduced engraftment of both human AML and CML stem cells.^{7,9} The inhibitory effects on IL-3 contributed to reduced AML engraftment and reduced AML LSPC survival, and prevented IL-3-mediated rescue of dasatinib- or nilotinib-induced CML LSPC death. Importantly, however, CSL362 directly mediated killing of leukemic cells via NK cells.^{7,9} These studies have prompted a phase I clinical trial of CSL362 in patients with AML (Clinical-Trial.gov identifier: NCT01632852).

We recently solved the crystal structure of a binary complex comprising a CSL362 Fab fragment bound to a soluble form of IL3R α (IL3R α :CSL362).¹⁰ This study revealed that the N-terminal domain (NTD) of IL3R α could adopt distinct conformations with respect to domains 2 and 3 (D2 and D3) of the receptor, with 2 non-identical forms of the IL3R α :CSL362 complex observed within the crystal. The NTD of IL3R α was positioned in either a “closed” conformation

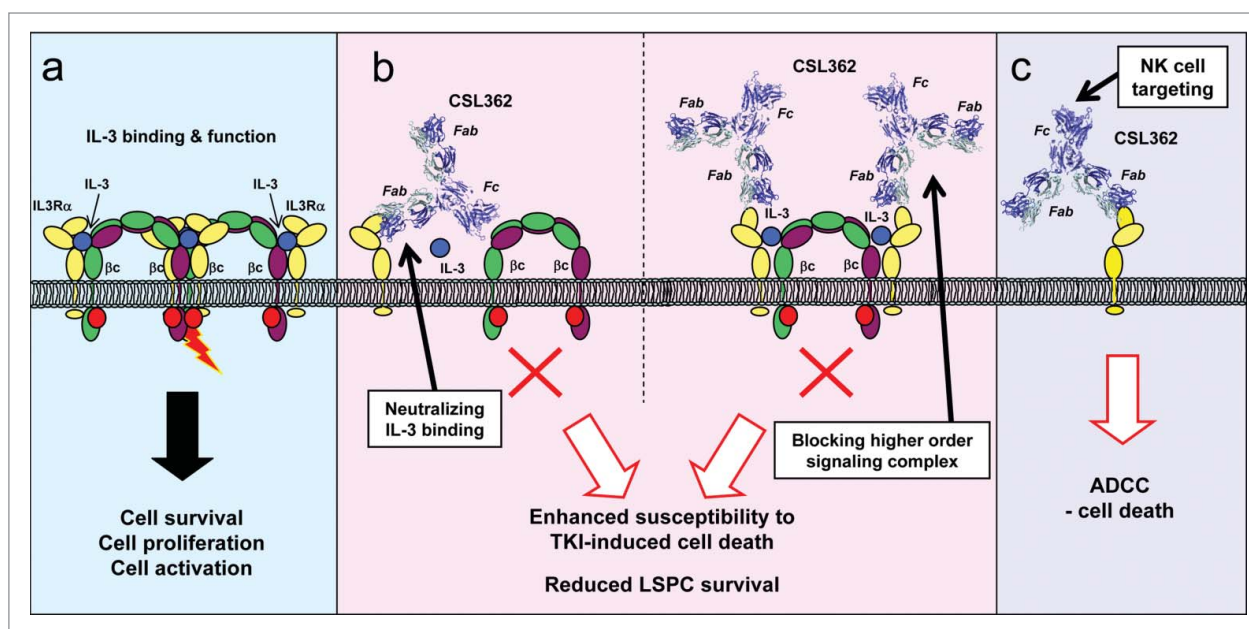


Figure 1. Dual mechanism of action of CSL362-induced cell death. The interleukin-3 (IL-3) receptor α subunit (IL3R α) is overexpressed on various leukemic cells, including leukemic stem and progenitor cells (LSPCs), and is associated with poor prognosis in acute myeloid leukemia (AML). (a) IL-3-mediated receptor signaling results in formation of a complex between IL3R α and the β common (β c) receptor (modeled on the granulocyte-macrophage colony-stimulating factor receptor structure²) that induces cell survival, differentiation, or proliferation. (b) CSL362 is a humanized anti-IL3R α neutralizing monoclonal antibody that has enhanced effector function and is currently in a phase I clinical trial in patients with AML. Neutralization of IL-3 signaling in leukemic cells enhances their susceptibility to tyrosine kinase inhibitor (TKI)-induced cell death and reduces the survival of LSPCs. CSL362 neutralizes IL-3-induced signaling by preventing IL-3 binding to IL3R α (left) and by preventing higher order signaling complex formation (right). (c) In addition to its neutralizing activity, CSL362 also has enhanced effector function that results in potent natural killer (NK)-cell mediated antibody-dependent cell-mediated cytotoxicity (ADCC) killing of target cells, including AML blasts, chronic myeloid leukemia (CML) blasts, and LSPCs.

or a novel “open” conformation. The “closed” conformation closely resembled the receptor conformation adopted by related cytokine receptor subunits, including the interleukin-5 receptor α subunit IL5R α and the interleukin-13 receptor α subunits IL13R α 1 and IL13R α 2, when bound to ligand. The main difference between the closed and open forms of the receptors was the angle between the NTD and D2, which showed a difference of approximately 40°. The open and closed forms of IL3R α observed in the crystal structure likely represent the dynamic extremes of potential NTD conformations. A disulfide bond between C76 in the NTD and C194 in D2 and a hydrogen bond between S74 in the NTD and D196 in D2 place constraints on the degree to which the NTD can open, whereas interactions between A72 in the NTD and F198 in D2 provide a “doorstop effect” to limit further closing of the IL3R α . Our data demonstrated that a likely consequence of the flexibility in the NTD is to allow subtle modulation

of ligand binding and subsequent downstream signaling. IL5R α , IL13R α 1, IL13R α 2, and the granulocyte-macrophage colony-stimulating factor receptor α subunit, GMR α , also have a flexible linker between the NTD and D2, suggesting a similar mechanism of receptor modulation.

Our crystal structure revealed that CSL362 binds exclusively to the IL3R α NTD through interactions involving the BC, DE, and FG loops and strand D of the NTD.^{8,10} Investigation of the functional IL-3 binding site in IL3R α through homology modeling and mutagenesis studies indicated that the binding interactions of CSL362 and IL-3 with IL3R α are largely non-overlapping, and prompted further analysis to understand how CSL362 is able to function as an antagonist of IL-3 function. In the closed IL3R α complex, binding of CSL362 prevents IL-3 binding through steric interference whereas in the open IL3R α complex CSL362 and IL-3 can potentially bind simultaneously. Using IL3R α mutations

that were proposed to force IL3R α to adopt an “open-like” conformation, we showed that CSL362 and IL-3 can simultaneously bind to an IL-3 receptor complex where IL3R α exists in an open-like conformation, but that the binding of CSL362 still blocks IL-3 receptor function.¹⁰ On the basis of these results we proposed that IL-3 signaling arises through the initial assembly of a 2:2:2, IL-3:IL3R α : β c hexamer complex that provides high-affinity IL-3 binding. Subsequent formation of a higher-order complex that requires interactions between hexamers allows β c-associated JAK2 activation and is essential for signal transduction. Such higher-order complex formation was first reported for the GM-CSF receptor and is potentially a common feature of the β c family of receptors.² Importantly, CSL362 can inhibit IL-3-induced receptor activation in either the classic closed form by directly blocking IL-3 binding, or in the novel open form by preventing higher order complex formation (Fig. 1b).

The dual mechanisms of CSL362-mediated IL-3 neutralization demonstrate quite distinct structural explanations that may be germane in understanding the blocking activity of other antibodies that block hormone receptor function.

Disclosure of Potential Conflicts of Interest

MWP and AFL are consultants for CSL Limited which is developing CSL362 and have received research support from CSL Limited. MPH and NJW are employees of CSL Limited.

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