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γ-secretase inhibitors: Notch so bad

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Leukemia treatment reveals safer side of γ-secretase inhibitors

γ-secretase inhibitors inhibit Notch, a transmembrane receptor which drives many cases T-cell acute lymphoblastic leukemia (T-ALL)—but there are safety concerns with such drugs. A combination treatment involving γ-secretase inhibitors and glucocorticoids could provide a more effective and safer approach.

A targeted combination therapy described in this issue of *Nature Medicine* may greatly improve outcome in individuals with a common childhood blood cancer: T-cell acute lymphoblastic leukemia (T-ALL)¹.

The therapy relies on the combination of a γ-secretase inhibitor with a glucocorticoid, dexamethasone. The γ-secretase inhibitor targets Notch1, a transmembrane cell surface receptor that regulates normal T cell development²; activating mutations in the gene encoding NOTCH1 cause more than half of TALL cases³. Dexamethasone counteracts lethal gut toxicity induced by the γ-secretase inhibitor. The authors outline how the combination therapy induces apoptosis in T-ALL cells—in T-ALL cell lines, primary human T-ALL cells and in xenografts of such T-ALL cell lines in mice—to a much greater extent than either dexamethasone or the γ-secretase inhibitor alone¹ (Fig. 1).

The approach, if they can be translated to people, might have benefits over current treatment of T-ALL, which accounts for 15% of childhood cases and 25% of adult cases of ALL⁴—a disease that affects approximately 5,000 people each year in the United States. Current multiagent chemotherapy regimens cure about 85% of children with T-ALL but only 50% of adults with T-ALL, and side effects are substantial⁵. The prognosis of adult patients has remained poor partly as a result of unfavorable genetic features of the leukemia as compared to those in children⁵.

NOTCH1 is a 2-chain molecule that, upon interaction of its extracellular domain with ligands of the Delta-Serrate-Lag 2 family, activates the intramembrane presenilin/γ-secretase protease complex that liberates the intracellular domain ICN1 of NOTCH1 from the lipid bilayer. After translocation to the nucleus, the ICN1 associates with the transcription factor CSL⁶ and activates the transcription of a set of target genes including the proliferation-promoting oncogene *MYC*. Although *MYC* upregulation contributes to NOTCH1-induced cellular transformation, experimental evidence suggests that the participation of additional responder genes is required in this process⁵. The introduction of activated *NOTCH1* alleles into mouse bone marrow leads to the development of T-ALL⁷. This finding suggests that NOTCH1 activation is also an aspect of the human T-ALL disease process. This hypothesis is further supported by the observation that activated NOTCH1 is essential for maintaining the transformed state of the human malignant cells⁸.

Real *et al.*¹ started their investigation by testing the hypothesis that T-ALL cell lines with activated NOTCH1 are resistant to glucocorticoid-induced apoptosis due to their aberrant NOTCH1 signaling. Indeed, the cells showed minimal loss of viability after treatment with dexamethasone or GSI alone but were highly sensitive to the combined treatment. The authors show that the direct target of activated NOTCH1, the inhibitory transcription factor HES1,

binds to the promoter of the gene *NR3C1*, which encodes the glucocorticoid receptor, thereby inhibiting its expression. Treatment with inhibitor enhanced *NR3C1* expression and activity, which up-regulates the expression of its known target, the proapoptotic gene *BCL2L1*, resulting in apoptosis (Figure 1).

How does dexamethasone reverse GSI-induced intestinal toxicity in mice? GSI causes severe intestinal secretory metaplasia as a result of marked increase in goblet cell differentiation⁹ and arrested cell proliferation in the intestinal crypts. The tumor suppressor gene *Klf4*, which encodes a Kruppel-like transcription factor, normally regulates goblet cell differentiation¹⁰. Again, the authors show that increased HES1 expression in GSI-treated mice suppressed *Klf4* expression directly by binding to the *Klf4* promoter¹ (Figure 1). Subsequent transcription profiling revealed that the intestines of mice treated with GSI and dexamethasone expressed more *Ccnd2*, a cell cycle regulator, than did that of mice treated with GSI alone. The authors provide evidence that the dexamethasone-induced expression of *Ccnd2*, at least in part, protects the mice from GSI-induced goblet cell metaplasia, though its observed effect on *Klf4* downregulation is indirect¹.

The known toxicity of dexamethasone treatment includes conditions such as osteopenia, hypertension and muscle atrophy; however, the potential effects of long-term inhibition of γ -secretase remain unknown. The enzyme γ -secretase targets more than 30 physiologically important transmembrane proteins, including the amyloid precursor protein involved in Alzheimer disease¹¹. Given that there are at least 6 different γ -secretase complexes in humans, subtype-specific inhibitors may be developed that have less broad side effects¹².

Clinical trials of GSI have been hindered by the considerable gastrointestinal problems experienced by the participants¹². Therefore, it will be necessary to determine whether GSI/dexamethasone combination therapy is as safe in humans as it appears to be in mice. No doubt it will take time to determine the optimal safe dosage for treatment; Real *et al.* report that in a small number of their mice, the combined treatment had to be stopped due to excessive weight loss¹. The combination treatment also increased lymphoid atrophy in the thymus and spleen, thereby potentially diminishing normal immunity.

Despite the promise of this approach for NOTCH-1 activated T-ALL, not all patients with this condition would be expected to respond. Eight percent of T-ALL samples harbor mutations in or show homozygous deletion of *FBW7*, a gene that encodes a ubiquitin ligase responsible for NOTCH1 ICN1 turnover. T-ALL cell lines carrying such mutations are resistant to GSI treatment¹³.

The findings of Real *et al.* may have broader implications, as NOTCH1 signaling is involved in many cancers¹⁴. By inference, the combination therapy might also be beneficial for solid tumors with aberrant NOTCH1 signaling. Likewise, GSI inhibits the production of amyloidogenic β -amyloid peptides involved in Alzheimer disease, and this work make provide new ways to treating that condition.

The new finding will initiate a flurry of research activity to further validate the combination therapy in additional preclinical studies¹. Provided that the outcome will be positive, the approach will be ready to move into phase 1 trials.

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Figure 1. Combined treatment of NOTCH-positive T-ALL with a γ -secretase inhibitor and dexamethasone

In untreated T-cells with mutant NOTCH (yellow dot), ICN1 released by γ -secretase associates with the transcription factor CSL and upregulates expression of the NOTCH target *HES1*. *HES1* binding to the promoter of the *NC3C1* gene inhibits its expression and results in insufficient production of the encoded glucocorticoid receptor- rendering these cells resistant to treatment with dexamethasone. Treatment with γ -secretase inhibitor and dexamethasone prevents the release of ICN and CSL remains associated with a repressor complex, inhibiting *HES1* expression. The inhibitor thereby restores autoinduction of the glucocorticoid receptor in the presence of dexamethasone and activates expression of its target genes, including

BCL2L11. Increased amount of the proapoptotic BIM protein induces apoptosis, which eliminates the NOTCH-activated T-cells.