comprehensive molecular characterization, as beautifully illustrated in the Read et al. study, along with similar definition of normal progenitor lineages should allow TPC categorization and ultimately a fuller understanding of the disease for design of individualized treatments.

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T Cell Acute Lymphoblastic Leukemia: NOTCHing the Way toward a Better Treatment Outcome

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γ-secretase inhibitors block the activation of NOTCH1 but have limited activity against T cell acute lymphoblastic leukemia (T-ALL) and cause severe gastrointestinal toxicity. In a recent study, Real et al. show that a potent γ-secretase inhibitor potentiates the cytotoxicity of dexamethasone against glucocorticoid-resistant T-ALL cells, while dexamethasone abrogates the gastrointestinal toxicity induced by the γ-secretase inhibitor.

T cell acute lymphoblastic leukemia (T-ALL), a clonal malignant disorder of immature T cells, represents 10%–15% of childhood and 25% of adult ALL cases. Although intensive chemotherapy has done much to improve prognosis for this disease, as many as 30% of childhood cases and approximately 50% of adult cases will relapse. Remarkable progress in understanding the genetic mechanisms underlying T-ALL pathogenesis has opened the way for the development of molecular targeted therapy. It is now clear that genetic abnormalities involving T cell receptor genes, basic helix-loop-helix genes (e.g., *TAL1*, *TAL2*, *LYL1*, *MYC*), cysteine-rich LIM domain-containing genes (*LMO1*, *LMO2*), or homeodomain genes (e.g., *HOX11/TLX1*, *HOX11L2/TLX3*, the *HOXA* gene cluster) can participate in the transformation of normal thymocytes by blocking differentiation. Abnormalities of a different group of genes (e.g., *CDKN2A/2B*, *CCND2*, *LCK*, *RAS*, *PTEN*, *ABL1*, *JAK2*, *FLT3*) appear to increase self-renewal, alter responses to extracellular signals, or impose resistance to apoptosis (Pui et al., 2008; Van Vlierberghe et al., 2008).

NOTCH1 encodes a heterodimeric receptor that regulates normal T cell development beginning as early as the commitment of multipotent hematopoietic progenitors to the T cell lineage (Figure 1). Activating mutations of *NOTCH1* represent one of the most common genetic abnormalities in T-ALL. Indeed, 60% of T-ALL cases possess such mutations (Weng et al., 2004; van Grotel et al., 2008; Asnafi et al., 2008). Among them, activating mutations of the *NOTCH1* heterodimerization domain or juxtamembrane extracellular region induce ligand-independent activation of the receptor. Truncating mutations of the COOH-terminal PEST domain of the intracellular region, on the other hand, extend NOTCH1 signaling by removing the Cdc phosphodegron domains and preventing the proteasome-mediated degradation of the intracellular domains of the receptor (Palomero and Ferrando, 2008). One of the proteins that binds to the Cdc phosphodegron and primes the intracellular subunit for degradation is the F box protein FBXW7. Thus, it is not surprising that mutations in *FBXW7* can also extend

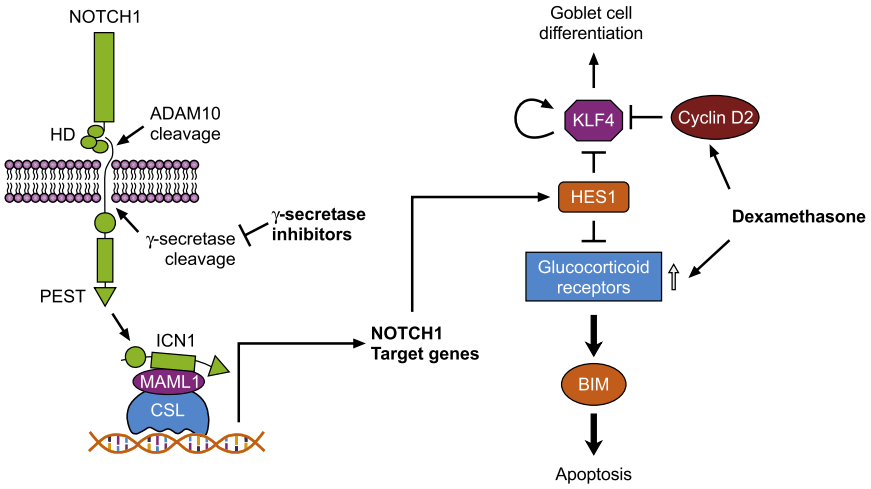

Figure 1. Schematic Representation of the NOTCH1 Signaling Pathway and Its Interaction with Dexamethasone Treatment

The mature NOTCH1 receptor is a heterodimeric single-pass transmembrane receptor consisting of a non-transmembrane extracellular subunit and a transmembrane intracellular subunit noncovalently joined via heterodimerization domains (HD). The receptors become activated when ligands of the Delta/Serrate/Lag2 family of proteins bind to the extracellular portion of the molecule. This interaction triggers a cascade of proteolytic cleavages, first by ADAM10 and subsequently by the γ-secretase complex, resulting in the release of the intracellular domain of NOTCH1 (ICN1). ICN1 translocates to the nucleus to form a complex with the CSL DNA-binding protein and the MAML1 (mastermind-like protein 1) transcriptional coactivator, leading to transcriptional activation of a diverse set of responder genes, including the *MYC* oncogene and the transcriptional repressor *HES1* (hairy and Enhancer of split homolog-1). NOTCH1 signaling is terminated through proteasome-mediated degradation of ICN1 after it is phosphorylated at the COOH-terminal PEST domain by CDK8. By blocking NOTCH1-HES1 signaling, γ-secretase inhibitors restore glucocorticoid receptor autoupregulation, resulting in effective upregulation of *BCL2L11*, which encodes a BH3-only protein (BIM) that has been implicated in glucocorticoid-induced cell death. Dexamethasone treatment induces upregulation of the cyclin D2 gene (*CCND2*) in the intestinal epithelium, which in turn inhibits the activation of KLF4 (Krüppel-like factor 4), a transcriptional factor controlled by NOTCH-HES1 signaling that functions as a negative regulator of the cell cycle required for goblet cell differentiation.

NOTCH1 signaling. Approximately 15%–20% of T-ALL cases have two coexisting lesions that activate NOTCH1 (Weng et al., 2004; van Grotel et al., 2008; Asnafi et al., 2008).

The precise mechanisms by which aberrant NOTCH1 signaling drives the pathogenesis of T-ALL are still unclear but probably entail constitutive expression of oncogenic responder genes, such as *MYC*, and activation of other signaling pathways (e.g., the phosphatidylinositol 3-OH kinase [PI3K]/AKT/mammalian target of rapamycin [mTOR] pathway and the NF-κB pathway) (Palomero and Ferrando, 2008). Because γ-secretase is required for NOTCH1 activation (Figure 1), inhibitors of this proteolytic step might abrogate the function of oncogenic NOTCH1 and suppress T-ALL cell growth. Indeed, γ-secretase inhibitors have been shown to induce cell-cycle arrest at G0/G1, decrease cell viability, and cause some apoptosis in a subset of T cell lines carrying *NOTCH1*-activating mutations (Weng et al., 2004; Lewis et al., 2007). However, clinical development of γ-secretase inhibitors for the treatment of T-ALL has so far been unsuccessful because of their limited antileukemic activity and their severe gastrointestinal toxicity resulting from massive conversion of proliferative intestinal crypt cells into postmitotic goblet cells in the gut (van Es et al., 2005; Deangelo et al., 2006).

Abandoning γ-secretase inhibitors as promising candidates for the treatment of T-ALL may be premature, as Real et al. (2009) present data in a recent issue of *Nature Medicine* indicating that these agents might be more effective and less toxic if they were given in combination with glucocorticoids. The authors found that a potent γ-secretase inhibitor (Compound E) and dexamethasone (a glucocorticoid widely used to treat T-ALL) have synergistic, though modest, activity against three glucocorticoid-resistant T-ALL cell lines with activated NOTCH1 and in two of three glucocorticoid-resistant primary human T-ALL samples. There was no evidence of such interaction in glucocorticoid-sensitive cell lines or primary leukemias, nor was there synergy between Compound E and antileukemic drugs of other classes. Interestingly, Compound E enhanced dexamethasone treatment-induced glucocorticoid receptor gene (*NR3C1*) autoupregulation and expression of the *BCL2L11* gene, which encodes BIM (Figure 1). The upregulation of *NR3C1* and the expression of BIM have been shown to be required for glucocorticoid-induced cell death. The authors further demonstrate the therapeutic efficacy of Compound E plus dexamethasone in leukemia-bearing mice.

Even if γ-secretase inhibitors do potentiate glucocorticoid killing of T-ALL cells, their gastrointestinal toxicity can still be an obstacle to their routine use in clinical settings. Real et al. counter this concern by showing that glucocorticoids can in fact protect the gut from the deleterious effects of NOTCH1 inhibition. This effect appears to be mediated by transcriptional upregulation of cyclin D2 and suppression of intestinal goblet cell metaplasia (Figure 1).

If the data of Real et al. are confirmed in additional T cell lines and primary clinical samples, how might γ-secretase inhibitors best be exploited in the clinic? Several studies have suggested that most T-ALL blasts with *NOTCH1* mutations respond well to glucocorticoid treatment. Among these, in the largest childhood study, the 82 patients with activating mutations had significantly better responses to single-agent prednisone treatment and more favorable long-term outcomes than did the other 75 patients without mutations (Breit et al., 2006). In addition, a recent study of 141 adult patients with T-ALL showed that the 88 patients with *NOTCH1* and/or *FBXW7* mutations had a slightly more favorable outcome than those without the mutations (Asnafi et al., 2008). These results indicate that the data of Real et al. may be applicable to only a subset of relapsed T-ALL patients with acquired resistance to glucocorticoid therapy. Thus, although NOTCH1 activation alone does not seem to induce glucocorticoid resistance, its inhibition might enhance glucocorticoid receptor function in very resistant cases, allowing the cells to respond to glucocorticoid treatment. In this regard, the use of high-dose dexamethasone significantly improved treatment outcome in patients with T-ALL in

a recent study (Schrappe et al., 2008). Should future studies demonstrate that the concomitant use of a NOTCH1 inhibitor with glucocorticoids can enhance the response of resistant leukemia, lower doses of dexamethasone could be used to treat such patients, sparing them from the toxicity of intensified chemotherapy. The protective effect of glucocorticoid therapy against the gastrointestinal toxicity of γ-secretase inhibitors might also renew interest in γ-secretase inhibitors as therapy for patients with Alzheimer's disease, for whom the drugs were first introduced to inhibit the production of amyloidogenic β-amyloid peptides.

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The Stem of Cancer

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Despite great advances in our understanding of tumor initiation and progression, the identity of the "cell of origin" of cancer remains elusive. Two recent publications provide experimental evidence that normal intestinal stem cells are the cells of origin of intestinal cancer in the mouse.

Colorectal cancer (CRC) represents a unique model to study mechanisms underlying tumor formation and progression. However, notwithstanding our detailed understanding of the initiating and rate-limiting mutation at the *APC* tumor suppressor gene and the subsequent genetic hits that accompany the adenoma-carcinoma sequence, the identity of the CRC cell of origin is still obscure.

The epithelium lining the gastrointestinal (GI) tract represents a unique stem cell niche where different cell types are spatially organized, each dedicated to a specific function. As recently shown by the Clevers laboratory, intestinal stem cells exist at the base of the crypts and, at least in the mouse, are earmarked by expression of the *Lgr5* gene in both the proximal and distal (colon) intestinal tract (Barker et al., 2007). These crypt base columnar cells divide about once per day, and their progenies, the transient amplifying (TA) cells, divide at an even higher rate and migrate up the crypt until they reach its midportion, where they differentiate into specialized intestinal functions. When the differentiated cells reach the top of the crypt-villus axis, they undergo apoptosis and are shed into the intestinal lumen. In such a hierarchical tissue architecture, it would be predicted that cancer cells would arise from (epi)genetic mutations in stem or early progenitor cells because of their long-term proliferation capacity and their ability to differentiate and acquire more specialized functions. However, an alternative model has been proposed in which the initial transformation occurs in an epithelial cell located in the intercryptal zone and in which the dysplastic process proceeds downward (Shih et al., 2001).

Two recent studies have provided evidence for normal intestinal stem cells as the cells of origin of intestinal tumors in the mouse (Barker et al., 2008; Zhu et al., 2008). Barker et al. bred a floxed *Apc* allele into the *Lgr5*-Cre mouse line, which expresses Cre recombinase controlled by the endogenous *Lgr5* locus, to selectively inactivate *Apc*, which leads to constitutive activation of the Wnt/β-catenin signaling pathway in the stem cells. Within days, these Wnt-activated stem cells generate transformed progeny that rapidly expand to the TA compartment. Eventually, multiple