Review

The Long Journey of *TCL1* Transgenic Mice: Lessons Learned in the Last 15 Years

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The first transgenic mouse of the *TCL1* oncogene was described more than 15 years ago, and since then, the overexpression of the gene in T- and B-cells in vivo has been extensively studied to reveal the molecular details in the pathogenesis of some lymphocytic leukemias. This review discusses the main features of the original *TCL1* models and the different lines of research successively developed with particular attention to genetically compound mice and the therapeutic applications in drug development.

Key words: *Lck-TCL1*; B-Cell; *Eµ-TCL1*; Chronic lymphocytic leukemia (CLL); Mouse models

INTRODUCTION

Twenty years ago, Giandomenico Russo and colleagues in our laboratory were studying the *TCL1* locus on human chromosome region 14q32.1 that was frequently involved in chromosomal translocations and inversions with one of the T-cell receptor loci in human T-cell leukemias and lymphomas (1). They eventually identified a gene coding for a 1.3-kb transcript, expressed only in restricted subsets of cells within the lymphoid lineage while expressed at high levels in leukemic cells carrying specific translocations or inversions within the chromosome regions 14q11 and 14q32. The *TCL1* gene, which is preferentially expressed early in T- and B-cell differentiation, was thus discovered (1). Later, *TCL1* was showed to be part of a new gene family of proteins involved in T-cell proliferations and leukemias, including T chronic lymphocytic leukemia (T-CLL) [reviewed in (2)]. Then, Narducci et al. characterized the murine *Tcl1* gene with the final aim to develop a mouse model of CLL, thus far the best way to decipher the role of the new oncogene in hematologic malignancies (3). The murine *Tcl1* gene was shown to reside on mouse chromosome 12, in a region syntenic to human chromosome 14, and to express early in mouse embryonic development, as well as in fetal hematopoietic organs and in immature T- and B-cells. Finally, to show that transcriptional alteration of *TCL1* was causally involved in the generation of T-cell malignant transformation, Virgilio et al. generated transgenic mice that carry the human cDNA for the *TCL1* gene under the transcriptional control of the p56 (lck) promoter element (4). The *lck-TCL1* transgenic mice developed mature T-cell leukemias after a long period of latency in old age, while younger mice presented preleukemic T-cell expansions expressing *TCL1* (4). In contrast to human leukemias, whose phenotype is, predominantly, CD4$^+$/CD8$^-$, murine leukemias are CD4$^-$/CD8$^+$. Thus, the *lck-TCL1* mouse, produced more than 15 years ago, was the first transgenic animal model to definitively prove the oncogenic role of *TCL1* in human leukemia and the initiator of a long line of studies with mouse models of leukemia that is thriving now more than ever. Immediately after the demonstration that *TCL1* overexpression causes mature T-cell proliferation in transgenic mice, expansion of *TCL1* analysis in many different types of human hematopoietic malignancies revealed strong expression of the gene in almost all tumor cells of B-cell lineage (5), thus indicating an important role in B-cell proliferation. Narducci et al. (6) confirmed that, in B-cell neoplasia, *TCL1* was expressed in the majority of the cases, including lymphoblastic lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, follicular lymphoma, Burkitt lymphoma, diffuse large B-cell lymphoma, and primary cutaneous B-cell lymphoma. Following these studies, times became mature to generate new models with *TCL1* expressed also in B-cells. Hoyer et al. (7) generated a murine model in which a *TCL1* transgene was expressed at similar levels

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in both B- and T-cells. These transgenic mice developed Burkitt-like lymphoma and diffuse large B-cell lymphoma at a very high penetrance beginning at 4 months of age. Only one mouse developed a T-cell malignancy at 15 months, consistent with a longer latency for transformation of T-cells by *TCL1*. These data demonstrated that, when overexpressed in both T- and B-cells, *TCL1* predominantly yields mature B-cell lymphomas (7). What about the *TCL1* overexpression in B-cells alone? The answer to this question was provided by the *Eμ-TCL1* transgenic mouse (8) (Fig. 1).

THE *Eμ-TCL1* TRANSGENIC MOUSE AND EARLY STUDIES

This animal model is characterized by exogenous overexpression of the human *TCL1* oncogene under the control of the a V(H) promoter–Ig(H)-*Eμ* enhancer to target gene expression specifically to B-cells and results in the clonal expansion of CD5$^+$/IgM$^+$ B-cells (8,9). Between 12 and 18 months of age, virtually all the mice develop an overt leukemia and massive infiltrations of monoclonal CD5$^+$ B-cells in both lymphoid and non-lymphoid organs, thus recapitulating the main features of human B CLL (or simply CLL). The *Eμ-TCL1* transgenic mouse was the first of a long series of engineered mice to develop a CLL-like disease but one of the very few to closely resemble the real human disease (9–11). Since its generation 12 years ago, the transgenic mouse has been widely used in the study of CLL pathogenesis and therapy. In the first study to exploit this model for potential treatment of CLL, we transplanted mouse CLL cells into syngeneic mice that led to a reliable and consistent development of the disease in the recipient mice (12). This approach allowed us to verify the involvement of the Tcl1/Akt/mTOR biochemical pathway in CLL by testing the ability of a specific pharmacologic agent, rapamycin, to slow the leukemia. Previously, in fact, the link between Akt and Tcl1, a coactivator of Akt, was demonstrated in vitro (13,14). The rapamycin-treated mice survived some time longer than the untreated ones but eventually succumbed to the disease. It was also demonstrated that the transformed murine lymphocytes, besides Akt, expressed other relevant therapeutic targets, like Bcl-2, and an in vitro sensitivity to therapeutic agents relevant to the treatment of human CLL (15). The same authors demonstrated also the in vivo clinical activity of fludarabine,
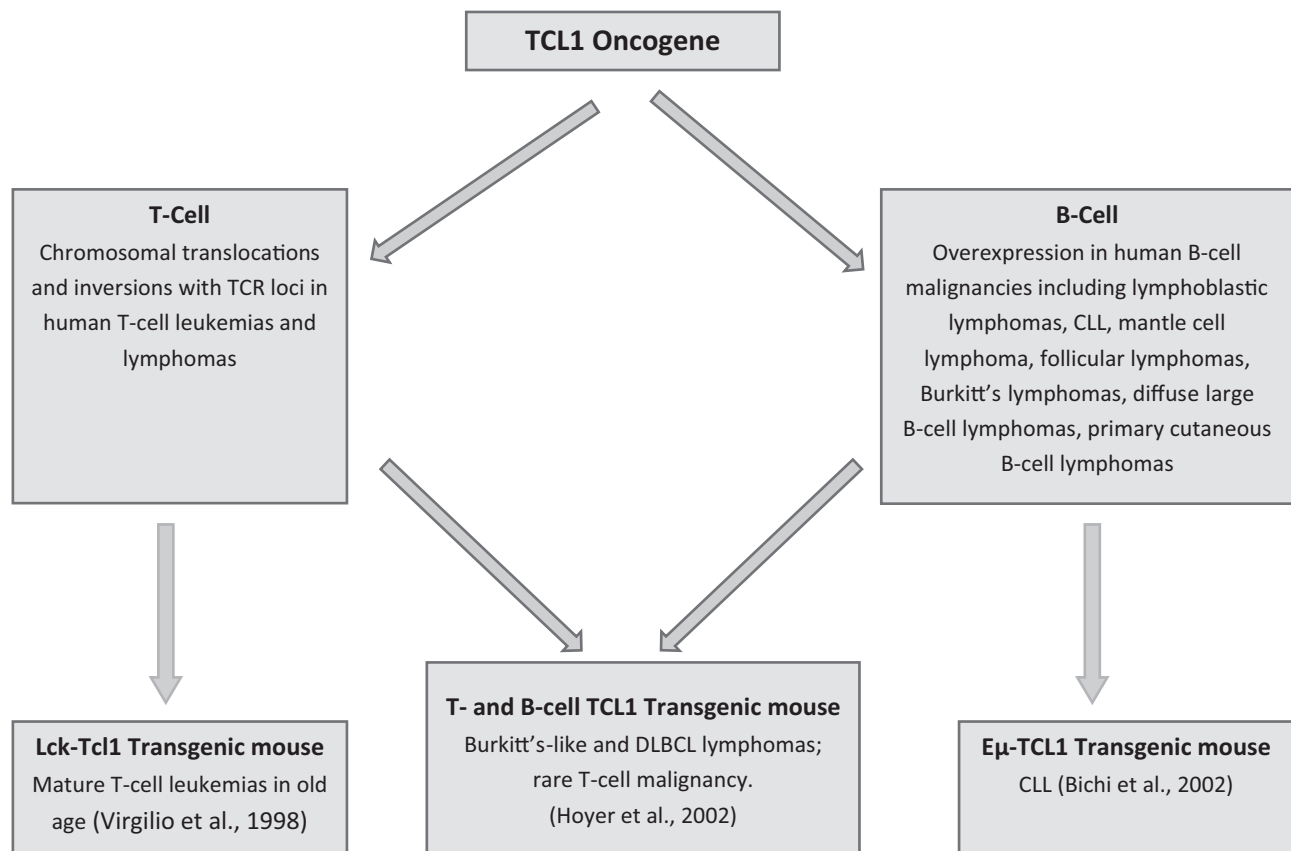

Figure 1. Generation of the first three TCL1 transgenic mice.

the classic CLL drug, in transgenic mice with active leukemia evidenced by early reduction in blood lymphocyte count, spleen size, and survival prolongation compared with control mice but, again, as in the case of rapamycin, an emergence of resistance put an end to the in vivo treatment (15).

The B-cell receptors (BCRs) expressed in *Eµ-TCL1* mice by lymphoid proliferation resembled those of aggressive, treatment-resistant human CLL (16). Protein kinase C β (PKCβ) is an essential signaling element of the BCR and has been shown to be overexpressed in human CLL (17). The laboratory of Alexander Egle used the *TCL1* transgenic mouse to directly target PKCβ in the development of murine CLL (18). *TCL1* overexpression did restore the CD5+ B-cell population that is absent in PKCβ-deficient mice. However, the compound PKCβ-deleted/TCL1 transgenic model did not develop a CLL disease, suggesting a role of PKCβ in the development of the malignancy and thus identifying a potential target for therapy (18) (Fig. 2).

THE *TCL1* MODEL OF CLL TAKES OFF

A similar approach to counteract the onset of CLL in *Eµ-TCL1* mice, but targeting a different pathway, has been adopted by Wu et al. (19). Signaling of the Wnt/Fzd pathway is known to play a key role in development,

tissue-specific stem cell maintenance, and tumorigenesis (20), particularly through stabilization of β-catenin, possibly affecting B-cell differentiation (21). To determine whether this pathway is involved in B-cell leukemogenesis, the authors examined the expression of Wnt/Fzd pathway genes in *TCL1* transgenic mice and found that, in the course of leukemogenesis, the expression of different genes, and most dramatically *Fzd6*, is progressively upregulated in the transformed CD5+ B-cells, as are protein levels of β-catenin. Interestingly, elimination of *Fzd6* expression by crossing into *Fzd6*−/− mice significantly delayed the onset of CLL in the compound animals. These findings suggest that self-renewal signals mediated by Wnt/Fzd, enlisted during B-cell development, may be pathologically reactivated in the neoplastic transformation of mature B-cells (19). Like rapamycin, silvestrol is another natural product that has been tested for its effects in *TCL1* transgenic mice (22). A plant-based agent with a unique chemical structure, silvestrol produces an unusual pattern of cytotoxicity that suggests activity in leukemia and selectivity for B-cells (23). In human CLL cells, silvestrol LC_{50} (concentration lethal to 50%) is 6.9 nM at 72 h, while in vivo, causes significant B-cell reduction in *TCL1* mice and other models identifying itself as a potential therapeutic agent for CLL and other B-cell leukemias (22). Immune-suppressive mechanisms that are
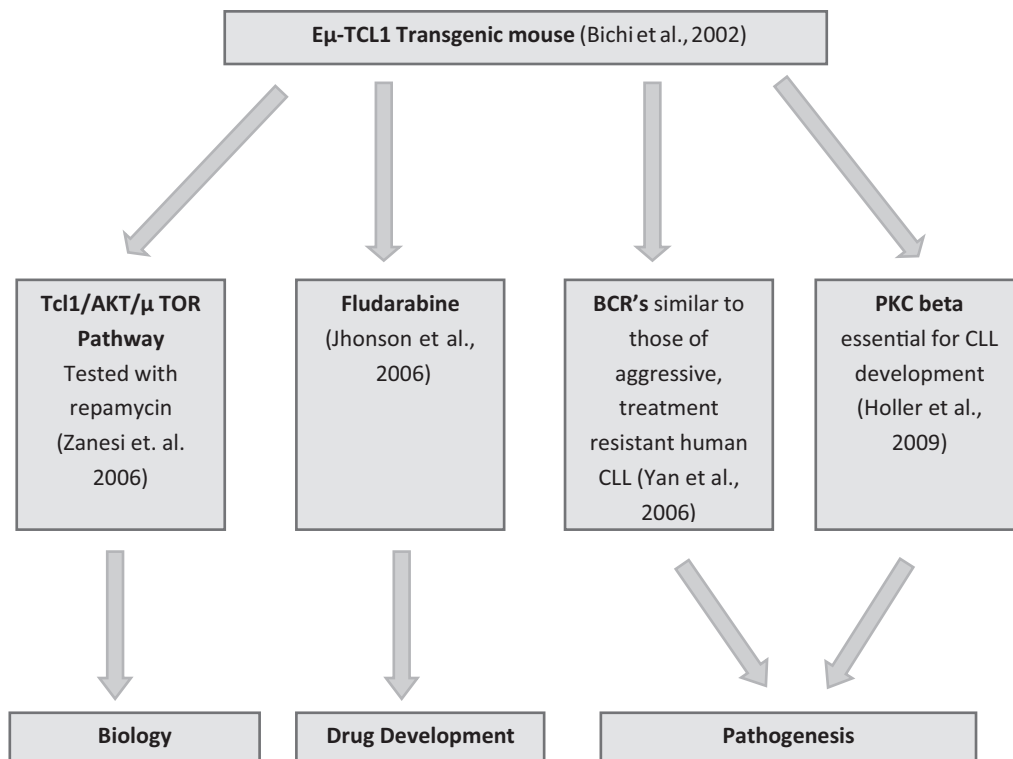

Figure 2. Early studies and main fields of investigation.

important in human cancers have been largely ignored in preclinical mouse models. In an effort to fill in this vacuum, John Gribben's laboratory examined the T-cell function in the *Eµ-TCL1* transgenic model (24). With development of leukemia, the mice also developed functional T-cell defects and alteration of gene and protein expression closely resembling changes previously seen in CLL human patients. Moreover, infusion of CLL cells into young, healthy *TCL1* mice induced defects comparable to those seen in animals with established leukemia, showing a causal relationship between CLL and T-cell defects. Altered pathways involved regulation of actin remodeling, dysfunctional immunological T-cell synapse formation, and T-cell signaling, which was reversed by the immune-modulatory drug lenalidomide. These results defined the *Eµ-TCL1* transgenic mice a versatile model to investigate both the molecular mechanisms of cancer-induced immune suppression and immunotherapeutic repair strategies (24).

A hallmark of nearly every malignancy, epigenetic investigations include gain or loss of DNA methylation (25). Different types of cancer-related genes can be affected by changes in DNA methylation, and because these changes are reversible, they are being extensively investigated as potential targets for therapy. Chen et al. (26) used the *TCL1* mouse to determine the timing and patterns of aberrant DNA methylation and to investigate the underlying mechanisms. Murine CLL cells were shown to recapitulate epigenetic alterations seen in the human counterpart. Aberrant methylation of promoter sequences was observed as early as 3 months of age in these animals, well before disease onset. Binding sites for the transcription factor FOXD3 were among the promoter regions abnormally methylated. It was demonstrated that loss of Foxd3 expression, due to an NF-κB p50/p50:HDAC1 repressor complex, occurs in *TCL1*-positive B-cells before methylation. Thus, specific transcriptional repression is an early event leading to epigenetic silencing of target genes in CLL. The results provide strong rationale for the development of strategies to target NF-κB elements in CLL (26).

CLL was once considered a disease characterized by an accumulation of noncycling B-cells, but studies based on heavy water labeling challenged this notion (27). Thomas Kipps and colleagues examined leukemia cell turnover in *Eµ-TCL1* transgenic mice and found that their leukemia cells not only had higher proportions of proliferating cells but also of apoptotic cells than did nonleukemic lymphocytes (28). Then, they crossed *TCL1*-Tg with *BAFF*-Tg mice, which express high levels of CD257. The derived compound mice, *TCL1/BAFF*, developed CLL-like disease at a significantly younger age and had more rapid disease progression and shorter survival than *TCL1* animals. Leukemic cells of double transgenic mice had similar proportions of proliferating cells, but fewer proportions of apoptotic cells, than did the CLL cells of *TCL1* mice. In addition, leukemia cells from double transgenic or *TCL1* mice produced more aggressive disease when transferred into *BAFF* mice than into wild-type animals. Neutralization of CD257 resulted in rapid reduction in circulating CLL cells. The results indicate that leukemic cells of *TCL1* mice undergo high levels of spontaneous apoptosis that is offset by relatively high rates of cell proliferation, which might allow for acquisition of mutations that, in turn, contribute to the evolution of the disease (28). The ζ-chain-associated protein kinase 70 (ZAP70) is a well-known prognostic factor in human CLL. RhoH is a hematopoietic-specific member of the Rho GTPase family that functions as a regulator of thymocyte development and T-cell receptor signaling by facilitating localization of ZAP70 to the immunological synapse (29). Sanchez-Aguilera et al. (30) investigated the function of RhoH in B-cell lineage. The BCR signaling was intact in *Rhoh*-KO mice. Because RhoH interacts with ZAP70, the mRNA levels of RhoH in primary human CLL cells were analyzed and showed a twofold higher RhoH expression compared with normal B-cells. RhoH expression in CLL positively correlated with the protein levels of ZAP70. By crossing *TCL1* transgenic mice with *Rhoh*$^{-/-}$ animals, the accumulation of CD5$^+$/IgM$^+$ leukemic cells in peripheral blood and the leukemic burden in the peritoneal cavity, bone marrow, and spleen of *TCL1*-Tg/*Rhoh*-KO mice were significantly delayed when compared with their TCL1 counterparts. Phosphorylation of AKT and ERK in response to BCR stimulation was also notably decreased in the double mutant splenocytes. Overall, the data suggest that RhoH has a function in the progression of murine CLL (30).

RECENT DEVELOPMENTS

As shown in Figure 2, the *Eµ-TCL1* transgenic mouse model has been used by many different research groups approximately along three main lines of investigation: a) biology, with attention to basic mechanisms of molecular biology and genetics; b) pathogenesis, to elucidate the mechanisms of CLL onset; and c) drug development, focused on testing novel therapeutic molecules. Here, we briefly summarize the achievements of the last 5 years along these lines of research.

Biology

An ever more detailed picture of interactions of *TCL1* with other genes and different pathways has been derived from the crosses of the *TCL1* mouse with other transgenic and knockout models. At least seven compound mice, involving four knockouts, *Hs1*$^{-/-}$, *p53*$^{-/-}$, *Id4*$^{+/-}$, and *Tir8*$^{-/-}$ (31–34), respectively, and three transgenic, the dominant-negative *RAG1*, *APRIL-TACI*, and *ROR1* (35–37), showed

phenotypes characterized by earlier onset of the disease and reduced survival compared to the single *TCL1* transgenic mouse. Two other compound animals, *TCL1/mif*$^{-/-}$ and *TCL1/CD44*$^{-/-}$ (38,39), on the other hand, displayed a later onset of CLL and longer survival. Therefore, from this rich panel of mutant combinations, we deduce that five genes, the intracellular protein hematopoietic cell-specific Lyn substrate-1 (*HS1*), the recombinant-activating gene 1 (*RAG1*), *p53*, through the p53/miR15a/16-1/Mcl-1 axis, the inhibitor of DNA-binding protein 4 (*ID4*), and *TIR8*, behave like tumor suppressors against the *TCL1*-induced leukemia. On the contrary, the other four genes, the macrophage migration inhibitory factor (*MIF*), a proliferation-inducing ligand (*APRIL*), the receptor tyrosine kinase-like orphan receptor (*ROR1*), and the cell-surface glycoprotein CD44 work to potentiate the oncogenic effects of *TCL1*. We generated a more complex compound mouse (40) based on the Sleeping Beauty transposon-mediated mutagenesis (41). This mouse genetic system had the aim to identify oncogenes that cooperate with *TCL1* and, through analysis of transposon common insertion sites, revealed that four out of seven genes identified were elements of the NF-κB pathway, thus confirming the importance of the cooperation between *TCL1* and NF-κB in CLL (40). We also generated a transgenic mouse using a construct containing the 3′ and 5′ UTRs of *TCL1* (full length) in B-cells (*Eμ-TCL1*FL). This model revealed accumulation of leukemic CD5$^+$/CD23$^+$ B-cells and increased levels of Akt phosphorylation, but overall, the phenotype was milder than in the classical *TCL1* mouse (42). From proteomic experiments to identify Tcl1-interacting partners, we found that Tcl1 protein physically interacts with de novo DNA methyltransferases Dnmt3A and Dnmt3B and, accordingly, B-cells from *TCL1* transgenic mice showed a significant decrease in DNA methylation compared with WT controls (43). Finally, TCL1 transgenic mice were also studied to compare the interleukin-10 (IL-10) competence and immunosuppressive function of CLL cells and regulatory B-cells. Malignant IL-10 competent CLL cells were found to exhibit regulatory functions comparable to normal regulatory B-cells that may contribute to the immunosuppression observed in TCL1 mice as well as in CLL patients (44).

Pathogenesis

Hofbauer et al. (45) investigated the composition of the T-cell compartment and observed a skewing of T-cell subsets from naive to antigen-experienced memory T-cells in *TCL1* mice, analogous to human CLL. Samson Jacob's research group, studying the truncated form of protein tyrosine phosphatase receptor type O (*PTPROt*), a tumor suppressor downregulated in CLL, found that a 60% decrease in *PTPROt* expression occurs in TCL1 mice at 7 weeks of age, and *TCL1* can repress the *PTPROt*

promoter by altering activating protein-1 (AP-1) element expression (46). Kriss et al. (47) described a novel mechanism of leukemic progression in these mice based on the activation of the endoplasmic reticulum stress response by *TCL1*. To elucidate host–tumor interactions, Mittal et al. (48) showed that *TCL1* mice display a high percentage of leukemic cells from the lymph node microenvironment with expression of key BCR and NF-κB molecules. Another pivotal step in mouse CLL B-cell activation and proliferation is the reciprocal stimulation between CLL cells and resident mesenchymal stromal cells like follicular dendritic cells (49).

Drug Development

Suljagic et al. (50) investigated the inhibition of BCR signaling with the selective Syk inhibitor fostamatinib disodium (R788) in *TCL1* mice that resulted in reduced proliferation and survival of malignant B-cells prolonging survival of treated mice. Among the p53-independent apoptosis inducers, actinomycin D was identified, tested in *TCL1* mice and reported to be more effective than fludarabine, probably since both prosurvival genes, *BCL2* and *MCL1*, are targeted by the drug (51). Another way to target the BCR signaling pathway in mouse CLL has been through the irreversible inhibition of Bruton's tyrosine kinase by ibrutinib, an orally bioavailable kinase inhibitor that has shown outstanding activity in human CLL. *TCL1*-induced leukemia was in fact delayed by ibrutinib administration (52). Finally, the same group of researchers, in John Byrd's laboratory, targeted another pivotal pathway to support CLL, the PI3K/AKT, with a novel molecule, the OSU-T315, whose AKT pathway disruption in CLL cell membranes of *TCL1* mice prolongs their survival (53).

CONCLUSIONS

The almost complete disease penetrance in the *Eμ-TCL1* transgenic mice, likely due to a strong oncogenic function of the human *TCL1* gene, which affects multiple pathways, including coactivation of AKT, activation of the NF-κB pathway, and inhibition of the activity of DNA methyltransferases DNMT3A and DNMT3B, is a unique case among all existing mouse models of CLL (11). Since the first publication in 2002 (8), the *Eμ-TCL1* transgenic mouse model of CLL has been put under the lenses of different fields in biomedicine, and several lines of research have come out among the most promising to pursue in the battle to know and tame this leukemia, such as compound genetic models, epigenetics, microenvironment, and three main biochemical pathways: BCR, AKT, and NF-κB. The deployment of this model has tremendously and logarithmically enriched the knowledge of the oncogenic activity of *TCL1* in the context of the in vivo studies of CLL. As of our writing, several more

investigations are going on with this model, some in the most cutting-edge fields of medicine.

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