Challenging Cutaneous T-Cell Lymphoma: What Animal Models Tell us So Far

Antonella Bresin¹, Elisabetta Caprini¹, Giandomenico Russo¹ and Maria Grazia Narducci¹

Cutaneous T-cell lymphomas are characterized by heterogeneity of clinical variants, further complicated by genomic and microenvironmental variables. Furthermore, in vitro experiments are hampered by the low culture efficiency of these malignant cells. Animal models are essential for understanding the pathogenetic mechanisms underlying malignancy and for discovering new anticancer treatments. They are divided into two main categories: those in which tumors arise in the host owing to genetic modifications and those that use tumor cell transplantation. In this review, we summarize the attempts to decipher the complexity of the pathogenesis of cutaneous T-cell lymphoma by exploiting genetically modified and xenograft models.


Cutaneous T-cell lymphoma pathophysiology

Cutaneous T-cell lymphomas (CTCLs) are non-Hodgkin’s lymphomas derived from the neoplastic transformation of T lymphocytes with cutaneous tropism. Clinically, CTCLs can be divided into two large groups: the indolent type and the aggressive entity (Olsen et al., 2011; Trautinger et al., 2017; Willemze et al., 2005). Mycosis fungoides (MF) is the most common type, with an incidence rate of 0.3–1 new cases per 100,000 United State population/year (Amorim et al., 2018; Willemze et al., 2005). Early MF represents low-grade lymphoma; however, it can progress to a more aggressive disease defined by involvement of the lymph nodes, peripheral blood, and internal organs. Sézary syndrome (SS) is a rare leukemic aggressive variant, accounting for 3% of all CTCLs, with a survival of 1–5 years or even longer if associated with low peripheral tumor burden at diagnosis (Alberti-Violetti et al., 2015; Kubica et al., 2012; Scarisbrick et al., 2015; Willemze et al., 2005). Immunophenotyping reveals a skin-resident effector–memory profile for MF cells and a central–memory profile for SS cells, suggesting a different tumor cell origin (Campbell et al., 2010); a high degree of intratumoral heterogeneity leading to the ultimate definition of phenotypic/functional plasticity has been recently described (Buus et al., 2018; Herrera et al., 2021; Horna et al., 2021).

The ideal animal model for CTCL

Similar to humans, some animals develop CTCL spontaneously, for example, horses, dogs, rabbits (Azuma et al., 2021; Huston and Quesenberry, 2004; Miller et al., 2015). Although these models may be ideal for studying etiology and disease progression, they are extremely rare. In contrast, mice do not show a spontaneous form of CTCL but are useful in studying the specific carcinogenic effect of one or more genes modulated in their background owing to lower costs and simpler logistics. An ideal model of MF/SS should be based on mature CD4+ T cells, with a predominant effector–memory (MF) or a central–memory (SS) phenotype. Malignant cells must at least infiltrate the skin, where they are in the activated state (Cristofoletti et al., 2019), but colonization of the blood, lymph nodes, and other visceral organs would improve the recapitulation of the disease.

Genetically modified mouse models

The main recurrent genetic lesions found in MF/SS affect the pathways of cytokine, Rb, p53, PTEN, ZEB1, Jak/signal transducer and activator of transcription (STAT) and NF-κB, chromatin remodeling, and DNA damage response (Caprini et al., 2018; Choi et al., 2015; Cristofoletti et al., 2013; da Silva Almeida et al., 2015; Iżykowska et al., 2017; Kiel et al., 2015; Vermeer et al., 2008; Wang et al., 2015). However, the contribution of each lesion and the molecular drivers of this disease have not emerged clearly. For example, PTEN or ZEB1 inactivation in mice causes lymphomas with an immature phenotype that do not infiltrate the skin (Hagenbeek and Spits, 2008; Hidaka et al., 2008; Tesio et al., 2016).

The microenvironment plays an important role in the pathogenesis of MF/SS (Herrera et al., 2021; Krejsgaard et al., 2017; Rubio Gonzalez et al., 2016). Chemokines and their receptors such as CXCL12–CXCRR4, CCL19/CCL21–CCR7, CCL17/CCL22–CCR4, CCL2–CCR2, and CCL27–CCR10 (Cristofoletti et al., 2019; Hu et al., 2014; Maj et al., 2015; Narducci et al., 2006; Picchio et al., 2008; Wu et al., 2020) or cytokines such as IL-2, IL-7, IL-10, IL-13, and IL-15 (Döbbeling et al., 1998; Geskin et al., 2015; Marzec et al., 2008b; Mishra et al., 2016; Wasik, 2015; Wu et al., 2019; Yamanaka et al., 2006) are directly involved in the survival, proliferation, and skin homing of malignant T cells. At the same time, nonmalignant cells (i.e., mast cells, tumor-associated macrophages, monocyte-derived dendritic cells) contribute to tumor growth and survival by producing these supporting factors and inhibiting the antitumor immune response (Berger et al., 2002; Rabenhorst et al., 2012; Schlapbach et al., 2010; Wilcox et al., 2009; Wu et al., 2014).

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Abbreviations: CTCL, cutaneous T-cell lymphoma; HDACi, histone deacetylase inhibitor; i.v., intravenously; MF, mycosis fungoides; miRNA, microRNA; PDX, patient-derived xenograft; PI3K, phosphoinositide 3-kinase; SCID, severe combined immunodeficiency; s.c., subcutaneously; SS, Sézary syndrome; STAT, signal transducer and activator of transcription
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### Table 1. Most Relevant Mouse Models Related to CTCL

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<td>STAT3 conditional overexpression</td>
<td>Early MF</td>
<td>CD4+-SP; CD44+, CD62L+</td>
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<td>Skin lesions reminiscent of Pautrier’s microabscesses; Massive lymphocytic infiltration of most lymphoid and nonlymphoid organs; Blood involvement</td>
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<td>Jak3-AV transduced bone marrow cells into C57BL/6 mice</td>
<td>CD8+ MF</td>
<td>Mixed population: CD8+ SP; CD44+, CD62L+, CD4+, SP; CD4+/ CD8- DP</td>
<td>Skin lesions reminiscent of Pautrier’s microabscesses; Massive lymphocytic infiltration of most lymphoid and nonlymphoid organs; Blood involvement</td>
<td>Need for bone marrow collection and transduction; polyclonal expansion; skin lesions more marked in secondary recipients, after long latency (5 months)</td>
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<td>Cell line xenograft</td>
<td>MF/SS</td>
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<td>PDX</td>
<td>MF/SS</td>
<td>Related to the patient</td>
<td>Genotype and phenotype of patients</td>
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Abbreviations: AV, A572V mutation; CTCL, cutaneous T-cell lymphoma; DN, double negative; DP, double positive; HDACi, histone deacetylase inhibitor; MF, mycosis fungoides; PDX, patient-derived xenograft; SP, single positive; SS, Sézary syndrome; STAT, signal transducer and activator of transcription; T RM, tissue-resident memory.

Interestingly, Mishra et al. (2016) found that hypermethylation within the IL-15 promoter prevents binding of ZEB1, causing a transcriptional increase in IL-15 in patients with MF/SS. The authors investigated the pathogenic role of IL-15 overexpression using a transgenic mouse model (IL15−tg). An initial peripheral expansion of NK cells and CD8+ T cells was followed by a transformation of large granular lymphocytes with a T cell–NK cell phenotype (Fehniger et al., 2001; Mishra et al., 2012). Moreover, a subsequent dermatological phenotype, similar to advanced MF, arose in 70% of the mouse colony (histology in Figure 3a on the original paper) (Mishra et al., 2016). As observed in human MF, CD8+ and CD4+ T cells expressing cutaneous lymphocyte antigen and CCR4, with a reduced expression of CD62L, infiltrated the dermis and epidermis (Table 1). The presence of erythematous plaques/patches and Pautrier’s epidermal microabscesses and moderate leukocytosis summarized advanced MF more than SS. On the other hand, atypical lymphocytes in blood smears with hyperchromatic and cerebriform nuclei resembled circulating Sézary cells.

The oligoclonality of the TCRVß repertoire entailed mixed subpopulations. In fact, IL15−tg T cells transplanted subcutaneously (s.c.) in severe combined immunodeficiency (SCID) recipients caused skin infiltration and peripheral expansion of CD3+CD4+CD8−T cells or an aggressive population with a CD3+CD4−CD8− clonal phenotype in different subgroups. The IL15−tg model was used to test the efficacy of different histone deacetylase inhibitors (HDACis) on the basis of the link between IL-15 overexpression, HDAC activity, and miR-21 induction. In addition, they described an oncogenic loop due to IL-15 overexpression, miR-29b downregulation, and upregulation of the chromatin targeting gene BRD4 as responsible for the progression of MF and drug resistance (Kohnken et al., 2018). BRD4, in turn, upregulated miR-214 (Kohnken et al., 2019), which has proved to be a potential therapeutic target. This model remains the best characterized MF model developed to date, reaching advanced stages and producing preclinical data.

A mouse model that overexpresses STAT3 in T cells by conditional gene targeting has also been developed.
(Fanok et al., 2018). These mice developed late lymphoproliferative disease with a skin phenotype visible around 8 months, including Pautrier microabscesses, similar to what is observed in early polyclonal MF (histology in Supplementary Figure S7c on the original paper). Activated/memory CD4 T cells (CD44hi/CD62Llo) populated lymph nodes other than skin and maintained a gene expression signature comparable with that seen in human MF (Table 1). Interestingly, by crossing this mouse with Rag2−/− OTII mice to narrow the TCR repertoire, thus avoiding its uncontrolled activation, the authors showed that TCR engagement and the presence of skin microbiota were critical factors for the pathogenesis and progression of MF. By reproducing early-stage MF, this model can be useful for studying pathogenetic mechanisms rather than for preclinical purposes.

Two other models are halfway between the genetically modified model and the xenograft (Table 1). On the basis of MYC overexpression (Vermeer et al., 2008) and INK4a/ARF (p16/p14) loss in MF/SS (Hwang et al., 2008), Adachi et al. (2015) showed that CD4+ murine T cells isolated from Ink4a/Arf−/− mice and transduced with the Myc gene produced erythroderma and histological features of MF when transferred to Rag2−/− mice (histology in Figure 4f on the original paper). The immunophenotype of these cells was comparable with that of MF cells, and their epidermotropism depended on hair follicle–derived IL-7, overexpressed in patients with MF. This model was aimed at studying the biology of resident T cells and would offer the opportunity to test therapies.

Finally, because the Jak/STAT pathway is one of the most involved in the pathogenesis of MF/SS (Krejsgaard et al., 2006; Marzec et al., 2008a), bone marrow cells carrying the constitutive expression of the Jak3 gene through lentiviral infection were intravenously (i.v.) transplanted into wild-type mice: this leads to a lymphoproliferative T-cell disorder with prominent skin infiltration (histology in Figure 6b on the original paper) (Cornejo et al., 2009). In this study, lymphocyte expansion involved a population of mature polyclonal CD8+ T cells, suggesting that the model could recapitulate the rare CD8+ subtype.

**Xenograft mouse model**

Xenograft-based in vivo models are widely used for the evaluation of new drugs in cancer research. The validity of this model has been confirmed by several studies that found correlations between preclinical data obtained in mice and clinical trials on patients (Johnson et al., 2001; Talmadge et al., 2007). Traditionally used for solid tumors (Steel et al., 1983), more recently, they have also been used for hematological disorders, including MF/SS (Table 1).

Nude mice are transplanted efficiently with MF cells (Bresin et al., 2020; Chen et al., 2009; Frei et al., 2008; Kremer et al., 2010; Küntzi et al., 2006; Li et al., 2020; Thaler et al., 2004; Tun-Kyi et al., 2008; Yano et al., 2007). SS cells require more immunocompromised mice such as SCID or the SCID-beige variant (Chang et al., 2012; Charley et al., 1990; Doebbeling, 2010; Han et al., 2012; Rosenblatt-Velin et al., 1997; Shao et al., 2010; Yano et al., 2007). NOD/SCID (Esmailzadeh et al., 2015; Han et al., 2018; Kamijo et al., 2018; Kato et al., 2016; Wang et al., 2018), NOG/NSG (Horwitz et al., 2018; Ito et al., 2009; Jain et al., 2015; Ng et al., 2018; Okada et al., 2019; Wu et al., 2014, 2015), or NOD/SCID-B2m−/− (Krejsgaard et al., 2010; Petersen et al., 2014; Raflkiaer et al., 2011).

The first mouse model based on MF-derived MyLa cells transplanted cells into nude mice (Thaler et al., 2004). After tumor formation, the cells were isolated and serially transplanted into other recipient mice to increase the take rate. This protocol was used also by others (Frei et al., 2008; Kremer et al., 2010; Küntzi et al., 2006; Thaler et al., 2004; Tun-Kyi et al., 2008). The first model based on SS-derived cell transplantation was described by Doebbeling (2010), who injected HuT-78 cells s.c. into SCID-beige mice with a take rate of 75% and a latency of 40–60 days. Later, NSG mice or the latest generation NOD CRISPR Prkdc Il2r gamma (NCG) strain were mainly used to transplant HuT-78 cells with greater efficiency (Huang et al., 2015; Jain et al., 2015; Kamijo et al., 2018; Li et al., 2020; Wu et al., 2014, 2015; Zhang et al., 2018).

Regarding the injection sites, subcutaneous transplantation is preferred for preclinical experiments because it is easy to perform and to measure tumor volume with a caliper during drug treatment; alternatively, intravenous route allows for dissemination studies.

Xenograft models were used to evaluate the antitumor effect of vorinostat (Krejsgaard et al., 2010) and other HDACis (Chen et al., 2009; Kato et al., 2016; Shao et al., 2010) as well as their relationship with microRNAs (miRNAs) such as oncosuppressive miR-150 (Abe et al., 2017) or oncogenic miR-155 (Moyal et al., 2017). Epigenetic dysregulation and aberrant levels of miRNAs are well-described features in MF/SS (Kohnken and Mishra, 2019; Manfè et al., 2013; Narducci et al., 2011), and xenograft models have contributed to the description of some deregulated miRNAs. The oncosuppressive activity of miR-150 was due in part to the down-regulation of the CCR6 chemokine receptor (Ito et al., 2014), whereas the increase in the level of miR-711 in mouse serum after engraftment of MyLa cells was related to chronic itching associated with MF through interaction with nerve fibers (Han et al., 2018).

The important success of immunotherapy in solid tumors has expanded this therapeutic opportunity also for the treatment of lymphomas (Weiner et al., 2021). The first evidence of anti-CCR4 antibodies for MF/SS immunotherapy was obtained using humanized NSG mice to mediate antibody-dependent cellular cytotoxicity to tumor cells (Ito et al., 2009; Yano et al., 2007). In addition, an increase in NK and reduced tumor infiltration of regulatory T cells has been described (Ito et al., 2009). Similar results were obtained with a different anti-CCR4 antibody, revealing the elicitation of the immune response even in mouse neutrophils (Chang et al., 2012; Han et al., 2012).

Kinase inhibitors have been extensively studied for MF/SS therapy through the xenograft model (Gallardo et al., 2018; Kittipongdaja et al., 2015; Kremer et al., 2010; Petersen et al., 2014; Wang et al., 2018; Zhang et al., 2018). Recently, we have shown the therapeutic potential of phosphoinositide 3-kinase (PI3K)/mTOR dual inhibitors in MF/SS on HH cells transplanted into nude mice (Bresin et al., 2020), with enhanced apoptosis induction in vivo but not in vitro.
Patient-derived xenograft

The patient-derived xenograft (PDX) model can be a powerful tool for translational research and precision medicine because reproducing a true-to-life system can lead to informative preclinical models for individual patients. These models maintain the gene expression profile and drug susceptibility of the primary tumor (Okada et al., 2019; Shi et al., 2020; Yoshida, 2020). Therefore, they are particularly useful when tumors have high intertumor and intratumor heterogeneity, as in the case of MF/SS, compared with diseases in which the dominant drivers are well-defined (such as BCR-ABL for chronic myeloid leukemia [Li et al., 1999], BCL2 for follicular lymphoma [Egle et al., 2004], TCL1 for T-cell prolymphocytic leukemia and chronic lymphocytic leukemia [Bichi et al., 2002; Bresin et al., 2016; Virgilio et al., 1998]) and for which transgenic or knockout models recapitulate human diseases with a high degree of fidelity.

Owing to the low take rate, primary cell engraftment requires murine strains devoid of functional T cells, B cells, and NK cells, precluding the study of the contribution of the immune response to therapies, a problem that could be overcome using humanized PDX models (ITO et al., 2009; Klicznik et al., 2020). On the other hand, M2-like macrophages are still present in NSG mice, and their role has been described as important not only in the engraftment of MF/SS but also in disease progression (Assaf and Hwang, 2016; Wu et al., 2014).

The first attempt to establish a PDX came from Charley et al. (1990) who transplanted subcutaneous fragments of a skin biopsy from a patient with SS in SCID mice. The malignant T cells were kept in the skin graft for a month but never spread outside. Twenty years later, Hoeller et al. (2009) injected i.v. cells derived from a patient with SS in C57Bl/6 mice to investigate the migratory behavior of SS cells in the skin. The study showed the role of the CCL17 and CCL27 chemokines in SS cell extravasation but not in the dermis.

Van der Fits et al. (2012) reported intrahepatic injection of SS cells into newborn immunodeficient mice, which resulted in a wide and highly variable spread into the dermis and many organs. However, it was not always possible to demonstrate the clonality of human CD3+ cells (van der Fits et al., 2012).

Recently, Townsend et al. (2016) have created a public PDX repository from patients with leukemia/lymphoma (www.proxe.org), including MF/SS. PDXs, also available at the repository, are those generated from Ng et al. (2018) with specific sequence alterations (TP53) or gene fusion (NPM-TYK2). Other authors also used PDXs from the repository for preclinical experiments, with the PI3K-γ/δ inhibitor duvelisib or drugs conjugated with an anti-ICOS antibody (Amatore et al., 2020; Horwitz et al., 2018) showing the potential usability of these compounds.

More recently, two interesting papers have detailed PDX from primary MF and SS cells. Wu et al. (2021) obtained a PDX by transplanting s.c. 6.6 × 10⁸ lymph node–derived cells of a patient with MF and two PDXs using cells from the blood of patients with SS injected i.v. in NSG mice. In MF-derived PDX, clinical signs occurred after 19 days. All mice developed erythematous skin lesions and dissemination into the spleen and other visceral organs, whereas peripheral circulation was found in one of the two SS-derived PDX. PDXs were propagated through serial transplants of cancer cells isolated from the spleen. Using this model, the authors obtained preclinical evidence of the therapeutic synergy between HDAC and PI3K inhibitors to inhibit tumor growth and induce apoptosis. In particular, the expansion of primary SS cells in mice allowed for the in vitro screening of a large number of compounds, revealing that inhibitors of the α and δ PI3K isoforms are the most effective.

A more complex method has been described by Poglio et al. (2021). PDXs were established by intrafemoral injection of 1 × 10⁶ SS cells into NSG mice, with a take rate of 13.6% within 9–18 weeks (Figure 1), and secondary xenografts were generated by subcutaneous injection of 1 × 10⁶ SS cells isolated from the primary PDX spleen, with a take rate of 100% within 5 weeks. In addition, long-term SS cell cultures from the spleen of primary and secondary PDXs were established. Permissive culture conditions were based on different cytokine supplements (mixture A: IL-2, IL-4, and phytohemagglutinin and mix B: IL-7 and IL-15) and were patient dependent, with no correlation with disease stage or tumor cell phenotype. Despite the clonality of TCRβ, the authors described the phenotypic heterogeneity of SS cells (naïve, central–memory, effector–memory cells, etc.), which further changed between pretransplant and post-transplant isolations. Interestingly, these data reflect the cellular plasticity observed in patients with SS and could represent the phenotypic changes related to functional status and to the differential expression of activation and proliferation markers between circulating and skin-resident cells (Buus et al., 2018; Herrera et al., 2021; Roelens et al., 2017). In addition, different subclones were detected using fluorescent in situ hybridization and comparative genomic hybridization analyses. Finally, the SS lines were used to compare the efficacy of different therapies currently used for the treatment of patients with SS (romidepsin, doxorubicin, and vorinostat), revealing a differential sensitivity to drugs among the SS lines derived from different patients.

Conclusions

The high intertumor and intratumor genetic and phenotypic heterogeneity of MF/SS has so far prevented the introduction of a single animal model that encompasses all the main clinical features of this disease, but this is also due to the difficult clinical peculiarities of the disease that populate the term of CTCL. However, thanks to the great efforts made by researchers; today, we have a range of models with different characteristics, which can be used according to experimental needs. The mouse models Il15-tg and STAT3 conditional are interesting tools to study the pathogenetic mechanisms of MF, even if less suitable for the more aggressive SS. Because the heterogeneity of hematological disorders described in Il15-tg mice and the prolonged time to tumor formation in STAT3 mice limit the use of these models in preclinical testing, the xenograft model using MF/SS cell lines is currently the most widely used for preclinical research. It allows for the use of MF or SS cells and offers some opportunities to study pathogenetic mechanisms and resistance to therapy.
On the other hand, although PDXs with primary MF/SS cells are challenging models owing to the low take rate and long wait times, with technical advances, they promise to progress toward preclinical models for personalized therapies. This is relevant in the case of diseases such as MF/SS, for which curative therapies are still lacking, and clinical trials are much more difficult to perform owing to the rarity of the disease and the increasing number of therapies available, which require increasing use of reliable preclinical models.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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