HCL2025: Expanding Research in Hairy Cell Leukemia to Better Characterize Its Biology, Develop New Therapies, and Optimize Outcomes for Patients

An Initiative of Hairy Cell Leukemia Foundation (HCLF) and The Leukemia & Lymphoma Society (LLS)

Statement on Future Research and Therapeutics for Hairy Cell Leukemia

Prepared by L. Greenberger, PhD on 10/27/20

I. Background

Hairy cell leukemia (HCL) is a rare hematological malignancy that occurs in approximately 1200 and 1400 new patients in the US and Europe, respectively (Kreitman, 2018; Gatta et al., 2011). Patients typically present with infections, splenomegaly, or the presence of cytopenias. However, asymptomatic patients can be found using routine peripheral blood analyses, as hairy cells are a distinctive feature of the disease.

Classical HCL (cHCL) and HCL variant (HCLv) are two forms of the disease based on the WHO 2018 classification. In cHCL, nearly 100% of patients have the V600E mutation (Tiacci et al., 2011) and express CD123+ and CD25+ markers (Table 1). In contrast, in HCLv, which comprises approximately 10-15% of the HCL population, patients do not have BRAF mutations nor typically express CD123+ and CD25+ markers. However, HCLv patients often have MEK mutations (Waterfall et al., 2014), and therefore, both types of disease have attributes reminiscent of “RASopathies.” Other characteristics of HCL are summarized in the table below. Remarkably, cHCL is typically found in 4 times as many men as women.

Table 1. From Polderdijk 2019

Table 1. *Characteristics of HCLc vs HCLv. Modified from Letendre & Doll* [13].

Characteristic		HCLc	HCLv
Age		Midlife	>60 years
Gender ratio (M:F)		4:1	1.6:1
CBC		Pancytopenia, Monocytopenia	Leukocytosis, Thrombocytopenia
Morphology		Absent nucleolus, more pronounced hair-like cytoplasmic projections	Prominent nucleolus, convoluted nuclei, absence of shaggy circumferential cytoplasmic projections
Splenomegaly		60–70%	85–100%
Bone Marrow Aspiration		Dry tap	Easy
BRAF-mutated		90–100%	0%
Immunophenotype	CD11c	+ + +	+
	CD20	+ + +	+ +
	CD22	+ + +	+ +
	CD25	+ +	-
	CD103	+ +	+
	CD123	+ +	-
	CD200	+ +	-
	FMC7	+ +	+ +
Cyclin D1 [14]		Overexpressed	Absent
Annexin A1		+	-
CD72		-	+

II. Treatments

The benefit of treatments for HCL should be judged based on efficacy, safety, and quality of life. Moreover, because patients can survive for many years, long-term outcomes need to be considered.

A. Response Rates of Traditional Therapies. The mainstay treatment for a naïve symptomatic HCL patient is the use of purine analogs (PNAs), either cladribine or pentostatin, which are highly and uniquely effective for this blood cancer. The overall response rates to PNAs for naïve cHCL and HCLv patients are approximately >95% and 44%, respectively (summarized in Kreitman et al 2013). The complete response rates to PNAs for cHCL and HCLv are 80% and 8%, respectively (Maitre et al., 2019; Robak 2011). These results underscore the high unmet medical need for HCLv patients. Based on a small trial, the addition of rituximab to cladribine in naïve HCLv patients induces a 90% CR rate (Kreitman et al., 2013), with a high rate of MRD negativity (Chihara et al., 2016).

B. Duration of response to Traditional Therapies. In general, the 10-year overall survival is 90% in patients treated with PNAs (Maitre et al., 2019). In a recent report following HCL patients beyond 10 years (in which the percent HCLv is undefined) the median overall survival was 27 years (Paillassa et al, 2020). Nevertheless, the cumulative 10-year relapse incidence was 39% and in patients receiving a second-line therapy the median relapse-free survival was 7 years. Beyond this, patients treated with PNAs have a 10-year cumulative incidence of solid and liquid tumors of 11 and 5% respectively; the latter liquid tumor occurrence is more than 6-times higher than the normal age-matched population (Paillassa et al, 2020). It is important to note that treatment of naïve HCLv patients with rituximab plus cladribine produced a median overall survival was approximately 2 years, which is clearly inferior to the result with cHCL patients on the same regimen (Chihara et al., 2016).

C. New therapies for HCL

1. B-RAF/ RAS pathway inhibitors. A major advance in the treatment of HCL was enabled by a technology that allowed wide scope, relatively rapid mutational analysis of tumors. Enrico Tiacci, M.D., University of Perugia, Italy (and subsequently other laboratories) demonstrated that nearly 100% patients with cHCL have a V600E BRAF mutation (Tiacci et al., 2011). BRAF, which is in the RAS signal transduction pathway, is a protein known to induce cancer. Moreover, LLS has funded additional work to show that other mutations are immediately downstream in the BRAF pathway. In particular, MEK mutations are found in approximately 0-22% and 38-42% of cHCL and HCLv, respectively (for summary see Maitre et al, 2019).

Since vemurafenib is an inhibitor of BRAF and is an approved therapy to treat melanoma patients with mutant BRAF, The HCLF and LLS funded Dr. Tiacci to explore the use of vemurafenib in HCL. He and others showed that vemurafenib partially controlled relapsed/refractory HCL (Tiacci et al., 2015; Dietrich et al. 2016). Vemurafenib produced a CR rate of approximately 40%. After 1 year, the median progress-free survival and overall survival was 73 and 91%, respectively (Tiacci et al., 2015). Nevertheless, MRD-negativity was rare, and relapse occurred frequently.

The HCLF and LLS continue to invest in new research and therapies via the LLS/HCLF grants to further improve outcomes for HCL patients. The first two trials are jointly funded by HCLF and LLS:

- **Enrico Tiacci, MD (University of Perugia, Italy**) is exploring if vemurafenib combined with either a MEK inhibitor or rituximab produces superior results compared to vemurafenib in relapsed/ refractory patients. The goal is to achieve long-term complete remissions.

- **Jae Park, MD (Memorial Sloan Kettering Cancer Center)** is studying vemurafenib in combination with obinutuzumab (a CD20-antibody that may have superior properties compared to rituximab), in naive patients with cHCL. In addition, the team is conducting genomic profiling of patient samples to understand treatment responses and disease biology. A trial with an ERK inhibitor (downstream of MEK) in patients with HCLv and relapsed classical disease is planned.

- **Robert Kreitman, MD (NIH)** is studying the combination of dabrafenib and trametinib, BRAF and MEK inhibitors, respectively, in relapsed BRAF V600E-mutated HCL patients. The confirmed ORR rate was 78% with a CR rate of 49%. PFS and OS rates at 12 months were both 98% (Kreitman et al., 2018).

2. CD22-toxin. As the work with vemurafenib was progressing, other approaches were paying off for HCL. In 2008, LLS funded Dr. Curt Civin (University of Maryland) who showed that a CD22-directed antibody – conjugated to *Pseudomonas* exotoxin A was very effective at killing acute lymphoblastic leukemia. This finding ultimately paved the way for FDA approval in 2018 for what is now called moxetumomab pasudotox (Lumoxiti®) to treat patients with relapsed or refractory HCL. Moxetumomab pasudotox is a recombinant immunotoxin targeting CD22, composed of a CD22-directed Ab fragment fused to Pseudomonas exotoxin PE38 (Kreitman et al 2018). Based on a single arm trial with 80 patients, the ORR and CR rates in R/R HCL patients was 75 and 41%, respectively. The duration of the response was not reached within the time of the study (median follow up

was 17 months). Most CRs were achieved without MRD-ve, which suggests that relapses in these patients are expected. Mild to moderate toxicities include capillary leak and hemolytic uremic syndromes, which can be prevented and managed conservatively. This agent was the first new FDA-approved agent to treat HCL in more than 20 years.

III. Continued challenges for the treatment of HCL

Despite this progress, our important work continues because:

- There is still no cure for HCL and relapses are the norm.
- HCLv patients often do not have durable response to treatment.
- Many patients die due to advancing disease, infections resulting from a compromised immune system, and/or second malignancies.
- The molecular basis of HCL is not well defined.

A. **Novel Therapies, Clinical Trials, and Long term outcomes**. As The HCLF and LLS look into the next 5 years, there is a clear need to improve treatment outcomes for patients with cHCL and HCLv. Combination therapy, like those listed above, are clearly needed and will likely provide incremental improvements in outcomes. Combination studies in the future are likely to include a multitude of other FDA-approved agents for B-cell malignancies (i.e. BTK inhibitors, BCL2 inhibitors, CD19-directed therapies). Beyond this, there may be an opportunity to avoid PMAs and the long-term side-effects associated with these agents.

As cHCL and HCLv are rare diseases, it is likely that trials will need to be conducted using a network of expert sites and be run as investigator-sponsored trials (ISTs). Pharma company support may be possible if the trials have the potential to register for approval by the FDA and/or EMA. It is noteworthy that an IST conducted by Dr. Steve Treon (DFCI) using ibrutinib for the treatment of relapsed Waldenstrom's Macroglobulinemia (68 patients) was sufficient for FDA-approval in this rare indication (Treon et al., 2015). Hence, small trials run as ISTs in rare cancers can enable regulatory approval.

The Clinical Trial Service Center (CTSC) and Information Resource Center (IRC) at LLS plays an important role in enhancing enrollment on trials. More information about the CTSC and IRC is found at LLS CTSC and LLS IRC, respectively. The worldwide centers of excellence established by HCLF are outstanding centers for referrals and treatment. These centers can be found at HCLF HCL Centers of Excellence.

Since many HCL patients have long periods of time between treatment and relapse, a registry of HCL patients that can be maintained for decades will be critical. The patient records, combined with their genomic analysis, are powerful sources of information to examine the molecular bases of response, identify rare side-effects, and understand long-term quality of life. HCLF and LLS are actively working on enhancing these resources for patients and researchers. The goal is that researchers in the future can take advantage of this rich source of information to learn more about the disease and treatment outcomes.

B. **HCL Biology**. There are many gaps in our knowledge about the biology of HCL that should be addressed. Many of these areas have been extensively explored for other blood cancers and have translated into the clinic as diagnostic markers, prognostic markers, and improved therapeutic outcomes. Therefore, these foundational studies in HCL are likely to lead to better therapies in the future, as they have for many other blood cancers. The following is a list of some of the major gaps in our knowledge for HCL:

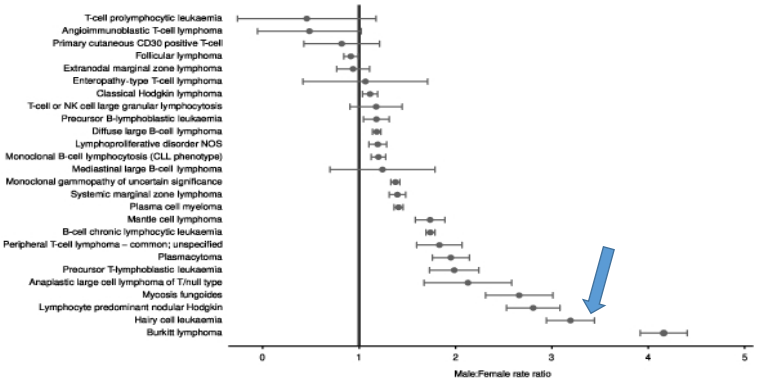
- **What is role of BCR signaling HCL?** The BTK protein is uniformly expressed in HCL, thereby suggesting that BCR signaling to NFκB could drive HCL. Consistent with this, the BTK inhibitor, ibrutinib, reduced the presence of HCL after the BTK pathway was activated by immunoglobulins (Sivina et al., 2014). It also inhibited CXCL12-induced signaling, which is a key pathway that controls homing to the bone marrow. It's possible that, like chronic lymphocytic leukemia, ejection of HCL cells from the bone marrow results in tumor cell death. This raises the question: what factors in the bone marrow support or possibly even promote HCL?

- **What explains relapses in HCL patients?** Relapses in HCL are common, albeit long after the treatment is concluded, suggesting that either a resistant (stem) cell exists or an independent event has occurred. Dr. Omar Abdel-Wahab and coworkers have already identified BRAFV600E mutations in the hematopoietic stem cells of HCL patients (Chung et al., 2014). This may suggest that HCL–HSCs are resistant to therapy and initiate relapse. This same work suggests that HCL cells are not derived from classical T- or B-cells because they have some markers of each type of cells, yet also have T- or B-cell markers that are not present from each cell type. If there is a unique stem cell for HCL, it may provide an index of pre-leukemic HCL or an early indicator of relapse. Beyond this, it seems possible that HCLv-cells have a distinct cell of origin compared to cHCL-cells.

- **What is the role of epigenetic alterations in HCL?** Little is known about the epigenetic changes that control gene expression driving HCL or leading to immune system evasion. Some of these genes are more frequently mutated in HCLv. As more epigenetic inhibitors are identified and explored in clinical trials for other blood cancer (Bates, 2020), these new agents may have utility in HCL.

  It was recently reported that, based on an epigenetic analysis of 11 HCL patients, the methylation signature of HCL is distinct from other B-cell tumors and involves genes regulated in the B-cell receptor as well as BRAF signaling pathways (Aribas et al., 2019). Frequent mutations in other epigenetic regulators including KMT2C, ARID1A, KDM6A, and CREBBP are found in HCLv (Maitre et al., 2019).

- **What explains the high prevalence of HCL in males?** Among HCL patients, the extraordinarily high ratio of men compare to women is remarkable (range 2.77 to 6.1; summarized by Polderdijk et al., 2019) and is the highest ratio compared to all other lymphomas except Burkitt lymphoma (Smith et al., 2010).



Ratio Male: Female. Blood cancers. From Smith et al. 2010

The causative basis for this is not understood. Notably, mutation in KDM6A are found in 12-50% of patients with HCLv, and this gene is located on the X chromosome. The role of this gene in the occurrence of HCL in men might be worth further study. Previous studies have suggested that the estrogen/ER axis may activate B-cells (Talaber et al, 2016). Is it possible that extraordinarily low estrogen levels in males could create an immunosuppressive environment that would allow the proliferation of HCL? Why would this apply to only HCL and no other lymphomas? Might we learn something by trying to understand why women get HCL?

- **What is the role of cyclin D in HCL?** Cyclin D is overexpressed in HCL. This raises the possibility that, like mantle cell lymphoma (Martin et al., 2019), response to CDK4/6 inhibitors may be obtained in HCL.

- **What is the molecular basis of the ruffled and ciliated appearance of HCL cells?** Very little is known about this, although it may be related to RHO GTPase interacting with actin. Since HCL cells do not maintain a ciliated appearance in vitro, is it is also possible that the tumor microenvironment influences the morphology? Single cell analysis of cells harvested from the patient's blood and bone marrow is a new technology that can help identify changes in the immune TME (Tikhonova et al., 2019) that may be applied to HCL. Comparisons between responding and relapsed patients are likely to be very informative.

- **Can novel immunotherapies control HCL, perhaps with a single treatment**? cHCL tumor cells have cell surface markers such as CD123, CD22, CD19, CD38, and CD25 which may be amenable to novel immunotherapies (i.e. CAR –T, bispecific antibodies, monoclonal antibodies, or antibody drug conjugates) that will specifically target HCL tumor cells with minimal toxicities. Notably, CD38 expression is a marker of poor prognosis in HCL (Poret et al., 2015). Since antibodies to CD38 (daratumumab or isatuximab), are safe and effective in myeloma, these antibodies, or CD38 – CAR T may be useful in the treatment of HCL. CAR-T cell therapy, using a single dose, has proven highly effective for certain leukemias and lymphomas (Majzner and Mackell, 2019). Such immunotherapies may also be useful in HCL, although there would have to be good justification for use of this expensive therapy. The expression of both T- and B- cell markers, which is unique to HCL, suggest that dual targeting of a T- and a B-cell marker may be particularly effective in HCL as it has been shown for other blood cancers (Qin et al, 2018). Exploration of other novel cell surface markers unique to HCL may also generate new targets for immunotherapy.

- **Can we develop additional in vitro and in vivo models of cHCL and HCLv?** New experimental models are needed, particularly for HCLv. There are almost no PDX models available for HCL. New models will help understand how HCL grows and will be useful in identifying new therapies worthy of clinical trials. Beyond this, HCL tumor cells do not retain their morphology outside the bone marrow, and therefore, are likely receiving survival signals in the tumor microenvironment (TME) within the bone marrow. Understanding the contribution of the bone marrow TME in HCL is likely to identify new targets to control HCL growth.

- **Will patient registries help define long-term outcomes and risks**? The longitudinal analysis of the progress of HCL patients treated with conventional agents suffers from

the lack of a large retrospective analysis of an annotated data set (that has electronic health care records and biopsy information), let alone a prospective database of patients that will be treated with B-RAF and MEK inhibitors. As patients can survive for decades, large databases with samples collected over long durations are needed to effectively conduct longitudinal analyses. These analyses may identify patients who are at risk of early relapse and therefore require more aggressive therapy. It is important to note that over 1,000 HCL patients and their caregivers participate in LLS's and HCLF's patient access programs. These resources provide unprecedented access to patients needed for this type of analysis.

To read about HCL2025, an initiative of HCLF and LLS: Expanding Research in Hairy Cell Leukemia to Better Characterize Its Biology, Develop New Therapies, and Optimize Outcomes for Patients, follow the link for the HCL2025 RFP.

IV. References

Arias. AJ et al., 2019. Genome-wide promoter methylation of hairy cell leukemia. Blood Adv. 3:384-396.

Arons, E et al., 2020. Expression of the muscle-associated gene MYF6 in hairy cell leukemia. PlosOne. 15:e0227586.

Bates, SE. 2020. Epigenetic therapies for cancer. N Engl J Med. 383:650-663.

Chihara, D et al., 2016. Long-term durable remission by cladribine followed by rituximab in patients with hairy cell leukemia: update of a phase II trial. Br J. Hematol. 174: 760-766.

Chung, SS et al., 2014. Hematopoietic stem cell origin of *BRAF*V600E mutations in hairy cell leukemia. Sci Transl Med. 6: 238ra71.

Dietrich, S et al, 2016. BRAF inhibition in hairy cell leukemia with low-dose vemurafenib. Blood.127:2847-2855.

Durham, BH et al., 2017. Genomic analysis of hairy cell leukemia identifies novel recurrent genetic alterations. Blood. 130: 1644-1648.

Gatta, G et al., 2011. Rare cancers are not so rare: The rare cancer burden in Europe. Eur J Cancer. 47:2493-2511.

Kreitman. R et al., 2013. Cladribine with immediate rituximab for the treatment of patients with variant hairy cell leukemia. Clin Cancer Res. 19: 6873-6881.

Kreitman. R et al., 2018. Moxetumomab pasudotox in relapsed/refractory hairy cell leukemia. Leukemia. 32: 1768-1777.

Kreitman, R et al., 2018. Treatment with combination of dabrafenib and trametinib in patients with recurrent/refractory *BRAF* V600E-mutated hairy cell leukemia (HCL). Blood 132: suppl 1: 391.

Maitre, E et al. 2019. Hairy cell leukemia: 2020 update on diagnosis, risk stratification, and treatment. Am J Hematol. 94:1413-1422.

Majzner, RG and Mackall, CL. 2019. Clinical lessons learned from the first leg of the CAR T cell journey. Nat Med. 25: 1341-1355.

Martin, P et al., 2019. A phase I trials of ibrutinib and palbociclib in previously treated mantle cell lymphoma. Blood. 133: 1201-1204.

Paillassa, J et al., 2020 Analysis of a cohort of 279 patients with hairy-cell leukemia (HCL): 10 years of follow-up. Blood Cancer J. 10:62.

Paillassa, J and, Troussard, X. 2020. Biology and treatment of hairy cell leukemia. Curr Treat Options in Oncol. 21:44.

Polderdijk, MCE et al., 2019. Deciphering the genotype and phenotype of hairy cell leukemia: clues for diagnosis and treatment. Expert Rev Clin Immunol. 15:857-867.

Poret, N et al., 2015. CD38 in Hairy cell leukemia is a marker of poor prognosis and a new target for therapy. Cancer Res. 75:3902-3911.

Qin H et al., 2019. Preclinical development of bivalent chimeric antigen receptors targeting both CD19 and CD22. Mol Ther Oncolytics. 6: 127-137.

Robak, T et al. 2010. Hairy-cell leukemia variant: Recent view on diagnosis, biology and treatment. Cancer Treat Rev. 37:3-10.

Sivina, M et al., 2016. The BTK inhibitor ibrutinib (PCI-32765) blocks hairy cell leukemia survival, proliferation and BCR signaling: a new therapeutic approach. Br J Hematol. 166:177-188.

Smith, A et al, 2010. The Haematological Malignancy Research Network (HMRN): a new information strategy for population based epidemiology and health service research. Br J Hematol. 148:739-753.

Talaber, G et al., 2016. Inhibition of estrogen biosynthesis enhances lymphoma growth in mice. Oncotarget. 7: 20718-20727.

Tiacci, E et al., 2011. BRAF mutations in hairy cell leukemia. N Engl J Med. 364:2305-2315.

Tiacci, E et al., 2015. Targeting mutant BRAF in relapsed or refractory hairy-cell leukemia. N Engl J Med. 373: 1733-1747.

Tikhonova, AN et al., 2019. The bone marrow microenvironment at single-cell resolution. Nature. 572: 222-228.

Treon, SP et al. 2015. Ibrutinib in previously treated Waldenstrom's Macroglobulinemia. N Engl J Med. 372: 1430-1440.

Waterfall, JJ et al.2014. High prevalence of MAP2K1 mutations in variant and IGHV-34 expressing hairy-cell leukemia. Nat Genet. 46: 8-10.

Wiber et al., 2019. Variant form of hairy cell leukemia. Clin Case Rep. 7: 1161-1166.