Rare, recurrent balanced translocations occur in a variety of cancers but are often not functionally interrogated. Balanced translocations with the immunoglobulin heavy chain locus (IGH; 14q32) in chronic lymphocytic leukemia (CLL) are infrequent but have led to the discovery of pathogenic genes including CCND1, BCL2, and BCL3. Following identification of a t(X;14)(q28;q32) translocation that placed the mature T cell proliferation 1 gene (MTCP1) adjacent to the immunoglobulin locus in a CLL patient, we hypothesized that this gene may have previously unrecognized importance. Indeed, here we report overexpression of human MTCP1 restricted to the B cell compartment in mice produces a clonal CD5+/CD19+ leukemia recapitulating the major characteristics of human CLL and demonstrates favorable response to therapeutic intervention with ibrutinib. We reinforce the importance of genetic interrogation of rare, recurrent balanced translocations to identify cancer driving genes via the story of MTCP1 as a contributor to CLL pathogenesis.
Chronic lymphocytic leukemia (CLL) is the most prevalent adult leukemia in Western countries and is characterized by a mature B-cell phenotype\(^1\). In contrast to other chronic adult leukemias, CLL is not primarily fusion/proto-oncogene driven. Instead, CLL pathogenesis likely begins with a lymphoid primed progenitor cell that becomes transformed at either a pre- or post-germinal center developmental stage bearing IGHV\(^\ast\) mutational status and a distinct epigenetic pattern corresponding to points along normal B-cell development\(^2\).\(^3\).\(^4\).\(^5\).

During CLL transformation, loss or gain of genetic material then appears to be a key determinant of disease phenotype and clinical outcome, with chromosomal aberrations such as deletions in regions of chromosomes 11, 13, or 17 (del(11q23), (del(13q14), or del(17p13), respectively), or a gain in copy number of chromosome 12 (trisomy 12) observed in up to 80% of patients\(^6\).\(^7\).\(^8\).\(^9\).\(^10\). Alternatively, balanced translocations, specifically those including the immunoglobulin heavy chain locus (IGH; 14q32) resulting in constitutive overexpression of various proto-oncogenes in the B-cell compartment, occur far less frequently\(^2\).\(^3\).\(^4\).\(^5\). Despite their infrequency, molecular profiling of these rare rearrangements have revealed broad importance of previously unrecognized coding or non-coding genes critical to the pathogenesis of CLL. Practical application of this strategy facilitated understanding the role of the anti-apoptotic protein BCL2 in CLL. While abundantly present in follicular lymphoma and diffuse large B-cell lymphoma (DLBCL), the t(14;18)(q32; q21) translocation—involving the IGH locus and the BCL2 gene—is a rare event in CLL (1–2% of cases)\(^9\).\(^10\). Yet even in absence of a t(14;18)(q32;q21) rearrangement, it was found that BCL2 mRNA was over-expressed in virtually all CLL patients compared to normal B-cells\(^11\). Work to determine mechanisms driving this abnormality later revealed microRNAs miR-15/16 as leading posttranscriptional regulators of BCL2 and loss of miR-15/16 as a result of 13q14 deletions substantially associate with elevated BCL2 expression in CLL\(^12\).\(^13\).\(^14\).

The pathogenic importance of this discovery subsequently led to a greater understanding of CLL disease biology and mechanisms resulting in the dramatic clinical activity demonstrated by the BH3-mimetic venetoclax\(^15\), ultimately contributing to the overall improvement in the therapeutic management of CLL.

Along this principle, we have identified a CLL patient with a previously undescribed \(t(X;14)(q28;q32)\) translocation, which leads to co-localization of the mature T cell proliferation 1 (MTCP1) coding region with the IGH locus, a situation analogous to the translocation of BCL2. Despite no known role in CLL, we found elevated MTCP1 mRNA expression in CLL cells compared to normal B cells and that increased MTCP1 expression in CLL patients portends poor outcomes to chemoimmunotherapy. Further interrogating this phenomenon we demonstrate the capacity for MTCP1 to initiate development of an aggressive murine CLL-like leukemia, revealing MTCP1 as a target for exploring the pathogenic mechanisms driving CLL. This discovery produces an in vivo model to investigate these unexplored mechanisms and to evaluate therapeutic strategies with optimal translatability to the clinical setting.

Results

\(t(X;14)(q28;q32)\) translocation identified in CLL. The Xq28 locus containing MTCP1 is bicistronic, encoding two distinct transcripts as a result of an ancient insertion event\(^16\). MTCP1 (previously termed p13 MTCP1) lies within the first intron of an unrelated gene CMC4 (previously termed p8 MTCP1), with the two genes’ distinct open reading frames preceded by a shared 5′ UTR (Supplementary Fig. 1A). Previous studies, using X-ray diffraction to estimate the crystal structure of both p13 MTCP1 and p14 TCL1A proteins\(^17\).\(^18\).\(^19\), describe a high degree of overlap between both amino acid sequence and 3-dimensional protein conformation—highlighted by an eight-strand beta barrel tertiary structure remarkably unique to this family of proteins (Supplementary Fig. 1B)\(^20\).\(^21\).\(^22\). Translocations involving the MTCP1 (Xq28) and T-cell receptor (TCRA/D) genes have been shown to induce constitutive MTCP1 overexpression and is a leukemic driving event in T-cell-lymphocytic leukemia (T-PLL)\(^23\).\(^24\).\(^25\).\(^26\).\(^27\).\(^28\). To date, MTCP1 on the Xq28 locus has not been implicated in B-cell leukemia or lymphoma.

To explore a potential role for Xq28 translocations in B-cell malignancies, we screened metaphase karyotypes of 1,744 cases suspected of CLL and identified eight (0.45%) with Xq28 rearrangements (Table 1). One case, a 59 year old female patient with IGHV-unmutated CLL, harbored a reciprocal \(t(X;14)\) (q28;q32) translocation, possibly involving the MTCP1 coding region and the IGH locus. To confirm this we performed fluorescent in-situ hybridization (FISH) analysis with probes directed against the IGH (3′ red; 5′ green) and MTCP1 (red) loci on metaphases. This showed that 5′ IGH moved to the X chromosome (Fig. 1A) and MTCP1 was indeed translocated to the 5′ end of the IGH locus (Fig. 1B). Combining the two probes demonstrated the MTCP1 and 3′ IGH probes co-localized on a chromosome 14, consistent with the results with MTCP1 by itself, and 5′ (green) IGH was on an X chromosome (Fig. 1C).

We then evaluated MTCP1 mRNA expression in CLL-B cells without any known Xq28 rearrangements and found ~2 fold higher MTCP1 mRNA transcripts in these CLL cells compared to naïve- or memory-B cells (Fig. 1D). A similar trend with CMC4 in CLL cells was also observed (Supplementary Fig. 1C). To interrogate the clinical significance of MTCP1 mRNA expression in CLL, we conducted a retrospective analysis on MTCP1 expression in CLL patients from two independent chemoimmunotherapy trial study cohorts for which microarray data have been previously reported (CALGB ’9712’ and ’1011’27).\(^28\). Here, we correlated CLL risk factors with MTCP1 expression using baseline characteristics obtained at time of treatment initiation (Table 2). When stratified into quartiles by MTCP1 expression we observed a similar distribution between sexes and high-risk CLL cofactors including age, performance status, cytogenetic evaluation, IgHV mutation status, and Zap-70 methylation; with the exception of elevated blood lymphocyte counts (WBC) and advanced Rai stage at diagnosis skewing towards patients with higher MTCP1 expression (Q2–4). As a single continuous variable, a 2-fold increase in MTCP1 expression was also found to associate with shorter progression-free survival (PFS) when adjusting for study cohort \((p = 0.03; \text{Supplementary Table 1})\). To illustrate this association, we plotted PFS by MTCP1 expression quartile for all patients and for each study cohort; visualizing that patients with the lowest expression (Q1) tended to have longer PFS (Fig. 1E; Supplementary Fig. 1D, E). Further, as IgHV mutation status is a strong predictor of outcome in CLL\(^3\), a bivariate analysis including continuous MTCP1 expression and IgHV status found a 2-fold increase in MTCP1 expression was prognostic for PFS independent of IgHV status \((p = 0.03; \text{Supplementary Table 2})\).
Table 1: Clinical demographics for suspected CLL cases with Xq28 rearrangements.

| Age (years) | Sex | Diagnosis | IGHV | Treated | Complexity | Karyotype* |
|------------|-----|-----------|------|---------|------------|------------|
| 59A        | F   | CLL       | Unmutated | No   | 2          | 47,XX,+12[17]/47,idem,t(X;14)(q28;q32)[2]/46,XX[2].ish t(X;14) (5'IGH+;3'IGH+) |
| 59         | F   | CLL       | ND    | Yes    | 3          | 46,X(t(X;12)(q28;q13))[3](t39;q28;q13.1),add(20)(q11.2)[9]/46,XX[10]/nonclonal[2].ish t(3;19)(BLCL+,BCL3+;BCL6–,BCL–) |
| 71         | M   | CLL       | Unmutated | Yes   | >6         | 46,XY,del(6)(q13)[cp2]/45,sl,der(6)(ins(6')?;?X)p21(2?)?del(6)(q21),der(8)t(8;15)(p21;q15),-15,del(16)(p11.2),add(17)(p11.2)cp19/45,X(t(X;12)(q28;q13))[3]-,+8,add(9)(q22),del(11)(p11),-22,cp21/46,XY[2]/nonclonal[1].ish add(17)(T/P53=--) |
| 74         | F   | CLL       | Mutated | Yes    | 4          | 46,XY,del(6)(q13)[2]/46,idiem,t(X;8)(q28;q22),inv(1)(q15)[3]cp3,one is 4n]/nonclonal w/ clonal abnormalities[1]/46,XX[16] |
| 59         | M   | Diffuse Large B-Cell Lymphoma | Unmutated | No   | >6         | 47,XY,der(X)(t(X;8)(q28;q24.2)ins(X;?)(q28;?)dup(1)(q24.2)dup(2)(q31)q35),t(3;9)(q7;9),del(7)(q22)del(9)(q21)del(9)X[8] |
| 60         | M   | CLL       | Unmutated | No   | >6         | 44,XY,del(9)(p22),psu dic(17)(p13q21),dic(18)(p11.2,p11.2)[11]/45,s,add(X)(q28),+add(9)(p22),-del(9)(p22),del(15)(21) |
| 64         | F   | CLL       | Unmutated | No   | >6         | 6-7<4n>XX,X-X,add(1)(q21)x2,add(3)(q29)x2,-4,-4,-6,-6,-8,-8,-9,-9,-13,-14,psu dic(17;5) inherits[3]t(12;13)(t(X;12)) |
| 56         | M   | CLL       | Unmutated | No   | 4          | 45,Y,der(X)(Xpter->Xq28;11ql2->11q13;1q32->1qter),der(1)(t(1;11)(q32;q33),der(11)?:11p15.5->11q12;Xq28->Xqter),21[cp2]/21/46,XY[16]/nonclonal[4].ish der(X)(CCND1+),der(1)(ATM+) |

*Xq28 rearrangement indicated in bold.  
**AFISH analysis of CLL cells from this patient are depicted in Fig. 1.

a transgenic mouse model expressing recombinant human MTCP1 under the control of a VH promoter-IgH-Eμ enhancer, targeting transgene expression to immature and mature B cells (Eμ-MTCP1; Supplementary Fig. 2A). Transgenic founders (Z36 and Z20) on the C57/BL6NTac background were bred to establish separate mouse lines, with successful passage of the MTCP1 transgene to progeny confirmed via PCR (Supplementary Fig. 3A). To further confirm transgene integration into founder mice, we performed targeted locus amplification30 in the established founder lines (Supplementary Fig. 2C, D). For both Z36 and Z20 founder lines, the genomic region between the 5′ and 3′ region of the integration site was deleted during the integration event. No structural variations were detected in the transgenic sequences, and >10 copies of the transgene integrated in each founder line.

CLL is characterized as an accumulation of CD45+ B lymphocytes with phenotypic expression of cell-surface markers CD5, CD19, and CD23, with dim—or intermediate—expression of CD45R (B220)4. To evaluate the potential of the Eμ-MTCP1 model to develop a leukemia resembling a CLL-like phenotype, littermate mice from Z36 and Z20 founder lines were followed monthly via flow cytometry analysis of the blood. Gating on CD45+ cells and probing for CD19+/CD5+ and CD19+/B220dim cell populations, a progressive expansion of circulating CLL-like B-cells were detected as early as 5 months of age in both Eμ-MTCP1 founder lines (Fig. 2A). The percentage of transgenic mice spontaneously developing a CLL-like leukemia were 70% and 28% for founder lines Z36 and Z20, respectively (Fig. 2B). Using detection of >20% CLL-like cells in the blood as a threshold for leukemia onset, Eμ-MTCP1 founder lines Z36 and Z20 reached “diseased” status at a median time of 7.7 and 16.7 months, respectively (Fig. 2C). Regardless of type and severity of hematologic abnormality, the majority of Z36 and Z20 Eμ-MTCP1 mice died spontaneously or met early removal criteria (ERC) due to evidence of clinical deterioration at a median time of 10.8 and 14.5 months, respectively—a significant reduction from the median lifespan observed in wildtype littermates (Fig. 2D, E). Notably, the progressive accumulation of CLL-like B cells in Eμ-MTCP1 mice followed a similar trend to that observed in Eμ-TCL1 mice (Supplementary Fig. 3A)—a widely used CLL mouse model which reliably (reported 95–100% penetrance) develops a CLL-like phenotype driven by overexpression of recombinant human TCL1 also under control of a VH promoter-IgH-Eμ enhancer31,32. A cohort of Eμ-TCL1 mice in our laboratory maintained a similar median time to CLL-like leukemia onset with Eμ-MTCP1 founder line Z36 and a similar median survival with Eμ-MTCP1 founder line Z20 (Supplementary Fig. 3B, C). Evaluating only mice that developed a CLL-like phenotype by censoring animals at the time at which a T cell or myeloid cell abnormality was observed, a competing risk assessment estimated the median survival for founder lines Z36 and Z20 to be 12.4 months and 17.6 months, respectively (Supplementary Fig. 3D). While the estimated median survival of Eμ-MTCP1 founder Z20 extended beyond that observed in Eμ-TCL1 mice (14.1 months), we observed a significant reduction in time from leukemia onset to death in both Z36 and Z20 Eμ-MTCP1 founder lines (4.10 months and 2.94 months, respectively), when compared to the Eμ-TCL1 model (7.41 months; Supplementary Fig. 3E). Taken together, this evidence suggests that while the rate of CLL-like leukemia onset in Z20 lineage mice...
MTCP1 driven leukemia recapitulates aggressive human CLL.

Eµ-MTCP1 mice meeting ERC due to CLL-like disease invariably presented with splenomegaly accompanied by abdominal lymphadenopathy (Fig. 3A). Histopathology evaluation revealed variable neoplastic infiltration of lymphoid tissues including robust splenic and lymphatic involvement with very modest presence in the marrow (Fig. 3B). Tumor cross sections exhibited variable degrees of F4/80 and B220-expressing infiltrates as detected by immunohistochemistry, with scant numbers of CD3+ mature, well-differentiated lymphocytes, which were interpreted as tumor-associated lymphocytes35. Healthy mouse tissues were infiltrated and effaced by neoplastic populations resembling both small-to-intermediate-sized lymphocytes and larger histiocytoid round cells, similar to those previously reported for the Eµ-TCL1 mouse model31.

A previously described hallmark of CLL B cells includes elevated expression of cytotoxic T-lymphocyte associated protein 4 (CTLA4)33. Our group has demonstrated that unless co-stimulation is provided, CTLA4 is not normally found on the surface of human CLL cells and expression is restricted to the intracellular compartment34. Using fluorescently labeled flow cytometry antibodies to detect both intracellular and surface CTLA4 in the blood from Eµ-MTCP1 mice, we found that, like human CLL and unlike Eµ-TCL1 CLL-like cells, CTLA4 expression was restricted to the intracellular compartment (Supplementary Fig. 3F).

MTCP1 driven leukemia recapitulates aggressive human CLL.

Fig. 1 MTCP1 is expressed in CLL patients and is associated with poor outcomes. A Metaphase FISH was performed on CpG-stimulated CLL cells using the IGH break apart (3′red; 5′green) hybridization probes. Representative FISH analysis in one CLL patient harboring the t(X;14)(q28;q32) translocation showing the IGH probe has split with 5′ (green) on an X chromosome and 3′ (red) remaining on a chromosome 14. Images represent three independent tests. Scale bars are 10 μm. B Metaphase FISH was performed on CpG-stimulated CLL cells using the MTCP1 (red) hybridization probe. Representative FISH analysis in the patient from (A) showing the MTCP1 loci are on an X chromosome and on distal 14q. Images represent three independent tests. C Metaphase FISH was performed on CpG-stimulated CLL cells combining the IGH (3′red; 5′green) and MTCP1 (red) probes. Representative FISH analysis in the patient from (A) showing the MTCP1 and 3′IGH probes co-localize on a chromosome 14 and 5′(green) IGH is on an X chromosome. Images represent three independent tests. D MTCP1 mRNA expression is elevated in CLL patients without any known Xq28 rearrangements compared to naive- or memory-B-cell subsets (p = 0.0012, p = 0.0013, respectively). Data obtained from the Blueprint database. Box elements reflect 2nd - 3rd quartile, center line reflects the median value, and whiskers reflect the distance from the upper and lower limit to the box elements. CLL cells - red/right, n = 7; naive B cells - dark gray/left, n = 9; memory B cells - light gray/middle, n = 5. P value estimated using a two-tailed unpaired t test with Welch’s correction. E Higher MTCP1 expression in CLL patients identified with shorter progression-free survival (PFS) in chemoimmunotherapy trials. CLL patients from two independent study cohorts from Cancer and Leukemia Group B clinical trials (9712 and 10101; N = 103) were retrospectively analyzed. Patients were divided into quartiles: Q1 (solid line, n = 25), Q2 (medium-dash, n = 27), Q3 (short-dash, n = 26), and Q4 (long-dash, n = 25) according to MTCP1 expression and PFS was visualized using the Kaplan-Meier method. As a continuous variable, the crude hazard ratio for a 2-fold increase in expression is 1.86 (95% CI: 0.97–3.55, P = 0.06) estimated from a Cox proportional hazards model. P value was determined via two-sided Wald test. No adjustments were made for multiple testing.
and CD21 expression were substantially increased in diseased Eµ-MTCP1 mice compared to wildtype littermates. Instead, atypical B cells lacking marginal zone progenitor cells were reduced in Eµ-MTCP1 mice but not wildtype littermates. Typically, lymphocytes like cells) with marked expansion in the blood was observed in Eµ-MTCP1 mice but not wildtype littermates. Usually, lymphocytes with varying degrees of IgM and IgD expression, pointing toward some heterogeneity within the bulk tumor population. Overall, the malignant cells of Eµ-MTCP1 mice showed a CD19+/CD5+/CD93−/B220dim phenotype with dim surface expression of IgM, IgD, and CD23, confirming a B1a cell phenotype. With respect to these surface markers, a similar trend between Eµ-TCL1 mice and wildtype littermates was observed.

Next, we analyzed the immunophenotypic signature of B-cell subsets using established murine markers of B-cell development. Representative plots are depicted in Fig. 3C, including comparison to blood from wildtype littermates and Eµ-TCL1 mice. After gating out CD3− T and CD11b+ myeloid populations, cells were visualized using CD19 and CD5 expression markers: a phenotypically homogeneous CD19+/CD21+ co-expression population (CLL-like cells) with marked expansion in the blood was observed in Eµ-MTCP1 mice but not wildtype littermates. Typically, lymphocytes of significantly diseased Eµ-MTCP1 mice were composed of >60% CD19+/CD5dim B cells. CLL-like cells and maturing B cells (CD19+/CD5−) were further dissected according to CD21 and IgM expression to identify CD21+/IgMdim marginal zone/marginal zone progenitor (MZ/MZP) B cells, CD21int/IgMdim follicular B cells, and CD21−/IgM− or CD21−/IgM+ atypical B cells. Among the CLL-like cells, the frequencies of follicular and marginal zone/marginal zone progenitor cells were reduced in Eµ-MTCP1 mice compared to wildtype littermates. Instead, atypical B cells lacking CD21 expression were substantially increased in diseased Eµ-MTCP1 mice. These CD19+/CD21− cells exhibited populations with varying degrees of IgM and IgD expression, pointing toward some heterogeneity within the bulk tumor population. Overall, the malignant cells of Eµ-MTCP1 mice showed a CD19+/CD5+/CD93−/B220dim phenotype with dim surface expression of IgM, IgD, and CD23, confirming a B1a cell phenotype. With respect to these surface markers, a similar trend between Eµ-TCL1 mice and wildtype littermates was observed.

### Table 2 Association between MTCPI expression and baseline characteristics in CLL patients.

| Expression (Log 2) | All Patients N = 103 | Quartile 1 Expression n = 25 | Quartile 2 Expression n = 27 | Quartile 3 Expression n = 26 | Quartile 4 Expression n = 25 | P value |
|-------------------|----------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|---------|
| Median            | 9.02                 | 8.52                        | 8.91                        | 9.11                        | 9.33                        | NA      |
| Range             | 8.16-10.26           | 8.16-8.70                   | 8.87-9.02                   | 9.03-9.19                   | 9.20-10.26                  | 0.28    |
| Study             | 9712                 | 41 (40%)                    | 7 (28%)                     | 9 (33%)                     | 12 (46%)                    | 0.07    |
| Age               | 10101                | 62 (60%)                    | 18 (72%)                    | 18 (67%)                    | 14 (54%)                    | 0.08    |
| Sex               | 12.8                 | 62                          | 55                          | 61                          | 66                          | 0.03    |
| Range             | 34-83                | 42-81                       | 34-77                       | 38-83                       | 46-79                       | 0.03    |
| Hemoglobin        | 5.5-16.9             | 5.5-15.1                    | 9.9-16.9                    | 8.2-15.5                    | 6.6-15.1                    | 0.66    |
| Median            | 108.6                | 67.0                        | 132.0                       | 108.6                       | 131.0                       | 0.47    |
| Range             | 6.3-436.0            | 6.3-238.0                   | 22.0-255.0                  | 29.0-402.0                  | 30.0-436.0                  | 0.47    |
| Missing/Unknown   | 3                    | 0                           | 1                           | 0                           | 2                           | 0.77    |
| Sex               | Male                 | 82 (80%)                    | 20 (80%)                    | 20 (74%)                    | 20 (77%)                    | 22 (88%) |
|                  | Female               | 21 (20%)                    | 5 (20%)                     | 7 (26%)                     | 6 (23%)                     | 3 (12%)  |
| Performance Status| 0                    | 61 (60%)                    | 14 (56%)                    | 12 (46%)                    | 18 (69%)                    | 17 (68%) |
|                  | 1/2+                 | 41 (40%)                    | 11 (44%)                    | 14 (54%)                    | 8 (31%)                     | 8 (32%)  |
| Missing/Unknown   | 1                    | 0                           | 1                           | 0                           | 0                           | 0.95    |
| Rai Stage         | I/II                 | 63 (61%)                    | 17 (68%)                    | 18 (67%)                    | 16 (62%)                    | 12 (48%) |
|                  | III/IV               | 40 (39%)                    | 8 (32%)                     | 9 (33%)                     | 10 (38%)                    | 13 (52%) |
| Cytogentic Group  | del(17p)/del(11q)    | 22 (24%)                    | 7 (30%)                     | 7 (30%)                     | 2 (8%)                      | 6 (26%)  |
|                  | Other                | 71 (76%)                    | 16 (70%)                    | 16 (70%)                    | 22 (92%)                    | 17 (74%) |
|                  | Missing/Unknown      | 10                          | 2                          | 4                           | 2                           | 2       |
| IgHV Usage        | Mutated              | 28 (31%)                    | 7 (30%)                     | 9 (38%)                     | 5 (23%)                     | 7 (33%)  |
|                  | Unmutated            | 62 (69%)                    | 16 (70%)                    | 15 (63%)                    | 17 (77%)                    | 14 (67%) |
|                  | Missing/Unknown      | 13                          | 2                          | 3                           | 4                           | 4       |
| Zap-70 Methylation| <20%                 | 80 (79%)                    | 19 (76%)                    | 21 (78%)                    | 20 (80%)                    | 20 (83%) |
|                  | ≥20%                 | 21 (21%)                    | 6 (24%)                     | 6 (22%)                     | 5 (20%)                     | 4 (17%)  |
|                  | Missing/Unknown      | 2                           | 0                          | 0                           | 1                           | 1       |
Fig. 2 Overexpression of MTCP1 drives a lethal CLL-like leukemia. A Longitudinal flow cytometry analysis of a representative Eμ-MTCP1 mouse showing progressive development of a CD45+CD5+/CD19+ and CD19+/B220dim CLL-like population in the blood. B Eμ-MTCP1 mice (Z36, n = 54; Z20, n = 36) were followed monthly by flow cytometry for disease progression as described in (A). Varying ratios of hemopathies were observed, including expansion of CD5+/CD19+ (CLL-like, pink) cells, CD5+/CD19− cells (T cells, orange), CD5−/CD19− (myeloid, green) cells, or CD19+/B220dim only CLL-like cells (tan). C Kaplan-Meier estimation of median time to disease onset in Eμ-MTCP1 mice (Z36 – red, n = 54; Z20 – blue, n = 36). Disease onset was defined as detection of >20% CD5+/CD19+ and CD19+/B220dim CD45+ cells in the blood determined by flow cytometry. Median time from birth to disease onset was shorter in Eμ-MTCP1 mice from the Z36 founder line (7.7 months) than from the Z20 line (16.7 months; p < 0.001). D Pie chart illustrating cause of death for Eμ-MTCP1 mice (Z36, n = 54; Z20, n = 36). Cause of death for a majority of Eμ-MTCP1 mice can be attributed to lethal progression of their disease burden. "ERC" = blue, mice met predefined early removal criteria. “Spontaneous” = purple, mice displayed disease progression identified by flow cytometry but had a spontaneous and unpredictable death. “Other” = green, mice died without measurable disease. E Kaplan-Meier estimation of median survival in Eμ-MTCP1 mice (Z36 - red, n = 54; Z20 - blue, n = 36). The median survival time was 10.8 months (95% CI: 9.5-12) for Eμ-MTCP1 founder line Z36 and 14.5 months (95% CI: 13.1-16) for founder line Z20 (p < 0.001). Representative survival of wildtype mice (black, n = 54) is shown as reference. P values (in C and E) determined by estimates from a Cox proportional hazards model.
bone marrow, Peyer’s patches, thymus, serosal surfaces of viscera; and rarely, lung and kidney (Fig. 4E). Thus, while a mixed lineage neoplasia was observed in Eµ-MTCP1 founder lines with a CLL-like leukemia, only the B-lymphoid portions of the total leukemic burden successfully engrafted and continued to proliferate in the host.

**CLL-like transcriptional profile identified in CD19^+CD5^+ populations from Eµ-MTCP1 mice.** To understand if the aggressive murine leukemia in Eµ-MTCP1 transgenic mice were composed of heterogeneous, polyclonal B cells or derived from a single precursor founding a homogeneous tumor population as is the case in human CLL, we evaluated the B-cell receptor (BCR) repertoire by examining IGH transcripts via RNA-sequencing methods previously described by our group36. Prominent usage of distinct heavy chain gene loci representing a clonal B cell expansion was observed from splenic B cells isolated from 3/3 Eµ-MTCP1 mice and similarly in 3/3 Eµ-TCL1 mice, a stark contrast from the high degree of clonal variability exhibited in wildtype

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**Fig. 3 Accumulation of abnormal B lymphocytes in Eµ-MTCP1 mice.** A Representative gross images of severely enlarged spleens from Z36 and Z20 founder Eµ-MTCP1 mice, an observation consistently observed in subsequent progeny comprising the Eµ-MTCP1 colonies. A representative gross image of a healthy spleen from an age-matched wildtype mouse is shown for comparison. Scale bar is 1 cm. B Post-mortem histopathology analysis of cervical lymph nodes, bone marrow, and spleen from Eµ-MTCP1 founder mice Z36 and Z20 with CLL-like disease show histologic changes in multiple tissues consistent with a systematically disseminated mixed neoplasm containing B-lymphocytes and histiocytes. Lymphocytes were typically B220^+, indicating a B-cell origin. CD3^+ lymphocytes were sometimes scattered throughout the neoplasm and may be tumor infiltrating lymphocytes. Histiocytoid cells were identified as F4/80 positive in most cases. Cells null for B220, CD3, and F4/80 were occasionally observed. Z36 images representative of n = 7 evaluated mice, Z20 images representative of n = 3 evaluated mice. All organs visualized at 60x, scale bars are 33.3 μm. C Representative immunophenotypic evaluation of B-cell populations in blood derived from Eµ-MTCP1 (Z20 at ERC, 9 months old), Eµ-TCL1 (at ERC, 12 months old) and wildtype mice (12 month old). CD3^+ T and CD11b^+ myeloid cells were gated out and cells were plotted using CD19 and CD5 expression markers to identify CD19^+CD5^+ population (CLL-like cells) and CD19^−CD5^− (maturing B cells). CD19^+CD5^+ population were further dissected according to CD21 and IgM expression to identify CD21^+IgM^+ marginal zone/marginal zone progenitor (MZ/MZP) B cells, CD21^+IgM^dim follicular B cells, CD21^−IgM^- and CD21^−IgM^+ atypical B cells. CLL-like cells were also evaluated for IgD and CD23 expression. Overall, the malignant cells of Eµ-MTCP1 mice showed a CD19^+CD5^+CD93^-B220^- phenotype with dim surface expression of IgM, IgD, and CD23 confirming a B1a cell phenotype.
mice (Fig. 5A, Supplementary Table 3). The major clone in 2/3 Eµ-MTCP1 and 3/3 Eµ-TCL1 mice predominantly used V_{11}1 and V_{11}12 family genes, while 3/3 wildtype mice predominantly used V_{11}5 family genes. One analyzed Eµ-MTCP1 mouse used V_{11}5 family genes in its dominant clones. The V_{11}12-3 gene was shared as that predominantly used in the major clone of one Eµ-MTCP1 and Eµ-TCL1 mouse. The most abundant tumorigenic clones isolated from Eµ-MTCP1 spleens exhibited a low IGHV mutational burden (Supplementary Fig. 4A), well below the threshold for classification \( ^{32} \) as “mutated” (Supplementary Fig. 4B). The low mutational burden in this region is consistent with the aggressive, IGHV-unmutated, subtype of human CLL.

We next sought to determine the overall transcriptional profile of these monoclonal tumor cells. Principal component analysis of global transcription profiles revealed significant segregation of splenic B-cells collected from Eµ-MTCP1 and Eµ-TCL1 mice, and splenic B-cells from wildtype littermates across PC1 (Fig. 5B). Further, a significant overlap in gene expression most variable from wildtype splenic B-cells was found when evaluating Eµ-MTCP1 or Eµ-TCL1 transgenic strains, suggesting the leukemic B1a cells in Eµ-MTCP1 mice have achieved a transformed state by both phenotypic and transcriptomic standards (Fig. 5C, D). Ingenuity pathway analysis (IPA) of significantly enriched genes (Log2FC > 2, \( p < 0.001 \)) shared between Eµ-MTCP1 and Eµ-TCL1 transgenic strains when compared to wildtype littermates primarily converged on genes involved in G protein-related signaling pathways (Supplementary Fig. 5A), likely a consequence from both Eµ-MTCP1 and TCL1 acting as activators of AKT. In genes uniquely enriched in Eµ-MTCP1 mice compared wildtype littermates, IPA analysis converged on FAK and PTEN signaling, Rho family GTPase signaling, and protein kinase A/cAMP-mediated signaling (Fig. 5E). Directly comparing Eµ-MTCP1 and Eµ-TCL1 mouse transcriptomes revealed a considerable degree of similarity, where only 79 of 15,318 analyzed genes displayed significant variation from one transgenic model to the other (Fig. 5F, Supplementary Fig. 5B). Specifically, 38 genes were identified as overrepresented in Eµ-TCL1 mice, highlighted by the presence of various nucleotide binding factors (Atrip, Ddx60, Trim6), heat shock proteins (Hspsa1/b), and other known oncogenic markers (Adm, Esf2k, Ilii2a, Ly6i, Mapk8, Map3k20, Pmaip1, Wnt16). Relative overexpression of 41 genes was noted in Eµ-MTCP1 mice, marked by the presence of cell signaling molecules (Ccr10, Lrp5, Zbtb4) and other known oncogenic markers (Cd34, Ptg1). Notably, no change in Tcl1 transcript abundance was observed between Eµ-MTCP1 and wildtype mice, and no change in Mtpc1 transcript abundance was observed between Eµ-TCL1 and wildtype mice (Supplementary Fig. 5C), suggesting the TCL1-driven murine leukemia acts independently from MTPC1 and the MTPC1-driven murine leukemia acts independently from TCL1.

**Ibrutinib treatment impairs Eµ-MTCP1 leukemia development.** We previously demonstrated the significance of constitutive activation of BCR signaling pathways in promoting proliferation of CLL cells, highlighting efficacy of the Bruton tyrosine kinase (BTK) inhibitor ibrutinib in abrogating leukemic advancement even in pre-leukemic stages\(^ {38} \). To evaluate the sensitivity of the MTCPI-driven murine leukemia to ibrutinib, Eµ-MTCP1 pups from founder line Z36 were treated continuously from the time of weaning with drinking water containing ibrutinib at \( \sim 30 \text{mg/kg/day} \) or 10% cyclocedrin (vehicle). Monthly assessment of circulating CD5\(^ + \)/CD19\(^ + \) and CD19\(^ + \)/B220\(^ {dim} \) leukemia cells in all treated mice displayed a lower leukemic burden at 6 and 12 months, contributing to considerable survival prolongation in mice receiving ibrutinib at 12 months of age (Fig. 6A, B). We further evaluated the use of the Eµ-MTCP1 adoptive transfer model for pre-clinical evaluation of CLL drug candidates. Successfully engrafted mice with comparable disease load (percent CD19\(^ + \)/CD5\(^ + \) B cells in the blood) were randomly assigned to receive ibrutinib or vehicle by daily oral gavage at any given enrollment time to control for different growth kinetics of the engrafted tumor cells. Similarly, ibrutinib administration led to a reduction in the rate of disease development between six and 12 weeks post-enrollment and prolonged survival compared to those receiving vehicle (Fig. 6C, D). Post-mortem histopathology analysis supported these evaluations, where ibrutinib-treated mice displayed less-severe organ involvement (Fig. 6E).

**Discussion**

Here, we identified an index CLL patient bearing an uncommon, reciprocal t(X;14)(q28;q32) translocation joining the MTCPI locus with immunoglobulin heavy chain regulatory elements (IGH; 14q32), an event analogous to the IGH translocations with MYC, CCND1, or BCL2 genes driving various B-lymphomas\(^ {39,40} \). After observing that this translocation occurs in CLL, we further discovered that even without an Xq28 rearrangement MTCPI mRNA is overexpressed in CLL cells as compared to normal B-cells. The observed overexpression of MTCPI in CLL and the discovery of a translocation that juxtaposes with the IGH locus suggest a pathogenically relevant role of MTCPI in CLL. Notably, increased MTCPI expression in this CLL cohort lacked major correlation with pre-treatment characteristics but was associated with a shorter response to chemoimmunotherapy. This relationship may reflect the current understanding of TCL1 expression in CLL, where inter-patient variability remains the norm yet patients with the lowest TCL1 expression maintain favorable outcomes following chemoimmunotherapy\(^ {41,42} \). Further correlating MTCPI expression with PFS, a bivariate analysis identified a 2-fold increase in MTCPI expression as a prognostic indicator of PFS independent of IghHV status, a known high-risk factor in CLL. Importantly, however, IghHV mutation status as a single variable was not prognostic for reduced PFS in this chemoimmunotherapy cohort—which is atypical in CLL—suggesting further large scale correlative studies are necessary to provide a comprehensive assessment of the associations between higher MTCPI expression and other high-risk factors in CLL.

In a multivariate analysis, a 2-fold increase in MTCPI expression lost strong association with reduced PFS while adjusting for additional predictors for reduced PFS in these chemoimmunotherapy trials (Zap-70 methylation, high-risk cytogenetics, sex, WBC). This result implicates MTCPI as a factor with considerable influence on the CLL disease course; yet it may not act as the fundamental driving element, which is not entirely surprising considering the abundance of evidence conferring the significant relationship between CLL outcomes and these other high-risk factors. Even so, revealing mechanisms supporting MTCPI upregulation in CLL may provide meaningful insight to the overall pathogenesis of this disease. Similar to studies evaluating TCL1 in CLL\(^ {43–45} \), the apparent inter-patient variability suggests multiple factors likely contribute to dysregulation of MTCPI expression; where loss of microRNA-mediated negative regulation or MTCPI upregulation via stimulating signals from the tumor microenvironment are primary candidates for further evaluation. Likewise, exploring BCR-induced kinase cascades, with particular attention given toward the role for PKC-\( \beta \)\(^ {46} \), may facilitate understanding of the upstream mechanisms upregulating and activating MTCPI in CLL. Other mechanisms supporting MTCPI upregulation, such as loss of epigenetic control or evasion of X chromosome inactivation (although we

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observed an even male:female distribution between *MTCP1* expression quartiles) serve as additional unexplored means facilitating *MTCP1* upregulation in CLL. In addition, exploration of this gene in other diseases such as acute myeloid leukemia (AML) might be considered based upon the recent identification of a t(X;17)(q28;q21) rearrangement resulting in a KANSL1-*MTCP1* fusion gene in an AML patient47.

Supporting the notion that *MTCP1* expression beyond basal levels is advantageous for leukemogenic B cells, screening a large cohort of suspected CLL cases revealed seven additional Xq28 rearrangements with unexplored relevance; two with a translocation at the 12q32 site, one each involving 8q22 or 8q24.2 sites, and three joining unrecognized material with the Xq28 site. Microdeletions in the 12q32 site including the HOXC cluster and
other adjacent genes results in significant disruption of normal cell function, and amplification of the 8q22 site including EDD1 and GRHL2 genes, negative regulators of apoptosis, have been described in breast, pancreas, and lung cancer cells as a mechanism for evasion of death receptor-activated therapies. The 8q24.2 region is a gene desert containing MYC enhancer elements and variations at this site are known to influence CLL. Interestingly, the (t(X;8)(q28;q24.2) case reported here was identified in a patient screened initially on the basis of suspected CLL, which was later found to be DLBCL. Cytogenetic evaluation of this patient, a 59 y/o male, also identified a t(3;9)(q27;q32) rearrangement involving the BCL6 gene and a t(14;19)(q32.3;q13.2) rearrangement involving the BCL3 gene. This t(14;19) rearrangement, joining the BCL3 locus with IGH elements, occurs in DLBCL and other chronic lymphoproliferative disorders but is most frequently observed in CLL. However, t(14;19) CLL cases are often atypical with distinctive clinicopathologic and genetic features including younger age, aggressive clinical course, and association with trisomy 12, which was also present in this patient. Translocation of the MTCPI locus under MYC enhancer elements in this unusual case supports our finding that, while rare, genomic rearrangement events resulting in up-regulation of the MTCPI gene may contribute to the transformative potential of the leukemic B cell and influence the overall trajectory of the resulting tumor burden. Juxtaposition of the MTCPI gene locus with these additional sites is of considerable interest and further investigation will be required to fully describe their significance. 

To establish a definitive role for MTCPI as a pathogenic contributor in CLL, we generated a mouse model with B cell-specific overexpression of human recombinant MTCPI (Eµ-MTCP1). Longitudinal evaluation of Eµ-MTCP1 littermates revealed a majority of these mice developed a lethal hematologic malignancy, highlighted by the progressive emergence of CLL-like B cells or hyperproliferative T cells circulating in the blood and accumulating in the spleen and lymph nodes. The CLL-like population in Eµ-MTCP1 mice bears a striking resemblance to the disease that develops in Eµ-TCL1 mice; however, the timeline for clinical deterioration from CLL-onset to death was accelerated in both Eµ-MTCP1 founder strains. The observed disparity in median estimated survival between Eµ-MTCP1 founder strains Z20 and Z36 appears to be largely driven by the delayed rate of leukemia onset in Z20 lineage mice. While we confirmed >10 copies of the MTCPI transgene were inserted in both founder lines, we acknowledge the integration site occurred at two distinct locations on different chromosomes for each respective founder. While mechanisms supporting the observed variance in leukemia onset between Z20 and Z36 founder lines remain unresolved, inserion of the MTCPI transgene in proximity to additional active enhancer regions or inaccessible in regions of condensed chromatin may be contributing to this distinction. Shared between founder lines, however, the cause of death for a majority of Eµ-MTCP1 mice was attributed to disruption of critical organ function due to systemic malignant infiltration comprised primarily of mixed populations of small-to-intermediate-sized lymphocytes and larger histiocytoid cells. Similar to what is often observed in CLL patients, the spleens of Eµ-MTCP1 mice were deformed, consistently presenting with substantial splenomegaly.

Further analysis of B lymphocytes from Eµ-MTCP1 mice revealed that this CLL-like disease may present a faithful resemblance to human CLL. We found that circulating CLL cells from Eµ-MTCP1 mice had intracellular, but lacked surface expression of the immunomodulatory molecule CTLA4, consistent with human CLL cells but not Eµ-TCL1 CLL-like cells. Immunophenotypic analysis of the CD19+/CD5− cells of Eµ-MTCP1 mice revealed the vast majority were B220low/CD93− expressing cells, suggesting a B1a cell phenotype. Scant numbers of IgM+ or IgD+ were found within this population, corroborating histopathology reports suggesting some degree of heterogeneity is evident within the malignant population. Engraftment of bulk splenocytes from Eµ-MTCP1 mice with CLL-like disease into immune competent hosts resulted in a homogeneous expansion of tumorigenic B lymphocytes, suggesting the true tumor population is comprised of the clonally related B1a cells and the chronic inflammatory milieu produced by the proliferating B cells in Eµ-MTCP1 mice may promote proliferation of macrophages, plasma cells, and extramedullary hematopoiesis.

Having demonstrated that the Eµ-MTCP1 mouse model generates a CD19+/CD5− B-cell malignancy with resemblance to human CLL, we proposed that this model may be ideally suited for pre-clinical evaluation of therapeutic agents for consideration in CLL and related diseases. To support this proposal, we demonstrated that continuous ibrutinib administration in pre-leukemic mice delayed disease onset and prolonged survival. In an adoptive transfer model, daily ibrutinib dosing delayed the rapid advancement of engrafted Eµ-MTCP1 splenocytes and resulted in a dramatic prolongation in survival. The moderate reduction in circulating lymphocytes upon treatment with ibrutinib in this adoptive transfer model is consistent with the understanding that inhibition of BCR signaling via ibrutinib drives cell mobilization from nodal sites and often results in prolonged lymphocytosis. The dramatic prolongation in survival seen here is likely a result of less severe occupation of proliferative lymphoid compartments and defacement of normal organ architecture in engrafted mice. Overall, the observed
response to inhibition of BCR signaling encourages further use of this model in development of novel derivatives with varying specificity for BTK and other components of the BCR pathway.

Although the overall transcriptome profile remained largely similar between Eµ-MTCP1 and Eµ-TCL1 tumor cells, further investigation to define the significance in variation between MTCP1- and TCL1-driven CLL is warranted. Specifically, elucidating the leukemogenic mechanisms which ultimately led to an accelerated disease course from CLL-onset to death in Eµ-MTCP1 mice is of considerable interest but is beyond the scope of the present study. Furthermore, the conserved structure between MTCP1 and TCL1A proteins and similarities in the resulting murine and human CLL phenotype suggest shared activation of AKT and other leukemogenic pathways; pathways which may be
independently activated as no observable change in Tcl1 expression was found in Eµ-MTCP1 mice and no observable change Mtcp1 expression was found in Eµ-TCL1 mice. Validation of this relationship remains unreported, however, for which the Eµ-MTCP1 mouse presents an excellent tool to study these mechanisms. The transgenic strategy of the Eµ-MTCP1 mouse presented herein also supports the notion that the CM4 gene is merely a passenger in the t(X;14)(q28;q32) rearrangement and may not provide an essential role in the leukemogenic transforming event. Regardless, CM4 expression was indeed elevated in CLL cells when compared to normal B-cell subsets. When translated to the short p8 Mtcp1 isoform this protein is localized to the mitochondria, likely suggesting an indirect role in supporting leukemogenesis via metabolic pathways. Thus, complete investigation of the relationship between p8 and p13 MTCP1 may further define the collective oncogenic impact of Xq28 rearrangements.

A broader and more general finding relevant to other types of cancer is the successful application of a strategy pursuing the functional consequence of genes involved in rare chromosomal abnormalities. Exploring the rare t(14;18)(q28;q32) translocation in CLL, such approaches facilitated understanding of the significance of the anti-apoptotic protein BCL2 and miR-15/16 at the minimal deleted region of del(13q14), likely suggesting an indirect role in supporting leukemogenesis via metabolic pathways. Thus, complete investigation of the relationship between p8 and p13 MTCP1 may further define the collective oncogenic impact of Xq28 rearrangements.

Methods

Screening suspected CLL cases for Xq28 rearrangements. A total of 1744 suspected CLL specimens collected between November 2003 and December 2014 at The Ohio State University Comprehensive Cancer Center were evaluated for possible Xq28 rearrangements by screening metaphase karyotypes collected at time of initial biopsy. Translocations involving Xq28 were confirmed by two independent cytogeneticists.

Mtcp1 and Cmc4 gene sequences were obtained and visualized from the Ensembl genome browser. The proposed 3-dimensional crystal structure for Mtcp1 and Tcl1A protein sequences were obtained from the RCSB Protein Data Bank (IDs: 1A1X, 1HSG, respectively). Crystal structures were determined via X-ray diffraction and presented at 2.00 Å and 2.50 Å resolution, respectively. Gene expression data for Mtcp1 and Cmc4 determined from RNA-sequencing were collected from the Blueprint DCC data portal. For evaluation of Mtcp1 expression via RNA-sequencing, de-identified human CLL cells were isolated as previously described after obtaining informed consent on protocols approved by The Ohio State University Cancer Institutional Review Board.

Fluorescent in-situ Hybridization. Fluorescent in-situ Hybridization (FISH) was performed with IGH/CCND1 XT, IGH break apart (Abbott Molecular, Downers Grove, IL) and Mtcp1 (Empire Genomics, Williamsville, NY) probes. FISH was done according to the manufacturer’s recommendations, except prior to hybridization slides were pretreated with pepsin and postfix solution. Co-denaturation of probe and sample was done on HyBrite (Abbott Molecular, Downers Grove, IL) at 3 min at 73°C. Hybridization was carried out overnight at 37°C, and slides were washed in 0.4 x SSC/0.3%NP-40 for 2 min at 73°C. The signals were viewed using a fluorescent microscope (Zeiss Axioscope 40) equipped with appropriate filters and analyzed with Applied Imaging System.

Generation of the Eµ-MTCP1 mouse model. Transgenic Eµ-MTCP1 mice were generated on a C57BL/6N background at The Ohio State University Comprehensive Cancer Center’s Transgenic Mouse Facility via pronuclear injection of linear constructs derived from a plasmid vector encoding murine immunoglobulin mu enhancer elements followed by human cDNA encoding the p13 kDa Mtcp1 protein. An equal ratio of male and female mice were maintained throughout all analyses.

From five unique transgenic founder lines, detailed characterization was conducted in two lines showing early evidence of a disease phenotype (Z36 & Z20). Genotyping of Eµ-MTCP1 progeny was performed using the following Mtcp1 primer sequences: (forward: 5′ ATCTGCGCCACATGGC 3′; reverse: 5′ GCT TAAAGCAAGCTCTCGTACT 3′). All experiments were carried out under protocols approved by The Ohio State University Institutional Animal Care and Use Committee. Pre-defined euthanasia criteria for mice in all transgenic colonies and murine transplant models included lethargy, impaired motility, splenomegaly, enlarged lymph nodes, decrease in body weight (>20%), development of tumor masses, ruffled fur, hunched back, failure to nest, and loss of appetite. All veterinary
technicians determining removal criteria were blinded to transgenic strain and
treatment group.

Transgenic mouse mapping and gene integration services were provided by
Taconic Biosciences via a collaboration between Taconic and Cergentis. Sample
preparation was carried out at Taconic Biosciences and both TLA and data analysis
were carried out at Cergentis as described previously30, with genotyping assay
recommendations subsequently provided by Taconic. The MTCP1 transgene
integrated at chr8:58,833,688–58,838,745 in an intron of the Galntl6 gene (Founder
Line Z20). The region in the genome between the 5′ and 3′ integration site was
deleted during the integration event. No structural variations were detected in the
transgenic sequence of this sample and >10 copies of the transgene have integrated
in this locus. Similarly, the MTCP1 transgene integrated at
chr7:100,715,866–100,720,642 within an intron of the Fam168a gene (Founder line
Z36). The genomic region between the 5′ and 3′ integration site was deleted during
Fig. 6 MTCPI-driven murine leukemia is responsive to BTK inhibition. A Continuous BTK inhibition via ibrutinib delays progression of a spontaneous leukemia in Eµ-MTCPI mice most evident by 12 months of age (CD19+/CD5+, p = 0.018, CD19+/-B220+/-, p = 0.012 determined via Cox proportional hazards model). Eµ-MTCPI mice (n = 13 per group) began continuous dosing of ibrutinib (~30 mg/kg/day, red line) or vehicle (10% cyclodextrin, black line) via drinking water (d.w.) beginning at two months of age. Plot reflects mean ± SE. B Mice from (A) were followed, revealing continuous BTK inhibition via ibrutinib (red line) prolongs survival compared to vehicle (black line, p = 0.006). **** represents estimation from a Cox proportional hazards model. C Adoptive transfer of 1×10^6 splenic Eµ-MTCPI CLL-like cells via tail vein to immune competent wildtype host mice results in a progressive leukemic expansion that is delayed upon treatment with ibrutinib. Mice were enrolled to receive either ibrutinib (red line, 25 mg/kg; n = 9) or vehicle (black line, 0.5% methylcellulose/1% Tween80; n = 9) via daily (Mon-Fri) oral gavage (o.g.) upon reaching ≥20% CD5+/CD19+ and ≥12% B220+/-CD45+ cells in the blood. Ibrutinib administration delayed the rate of leukemic progression measured at six (p = 0.063) and 12 weeks (p < 0.001) post-engraftment. P values determined via mixed effects model. Plot reflects mean ± SE. D Median survival time for mice in (C) is significantly extended upon daily administration of oral ibrutinib (red line, median survival = 28.4 weeks), extending well beyond the median survival observed with vehicle treatment (black line, median survival = 11.4 weeks; p < 0.001). **** represents estimation from a Cox proportional hazards model. E Post-mortem histopathology analysis of organs and tissues from mice in (C) receiving either ibrutinib (top panel) or vehicle (bottom panel) having succumbed to disease. Hematoxylin & eosin staining reveals extensive neoplastic infiltrates effacing the bone marrow and lymph nodes in both ibrutinib and vehicle treated mice, while infiltrates are less severe in the liver and spleen of mice treated with ibrutinib. Neoplastic cells are observed as discrete, basophilic nodules in the livers (arrows) and spleens (circled regions) of the ibrutinib-treated mice, whereas in the vehicle-treated mice, neoplastic cells tended to form diffuse sheets or larger/condensed nodules. All organs visualized at 10x, scale bars are 200 μm.

the integration event. No structural variations were detected in the transgenic sequence, and >10 copies of the transgene have integrated in this locus.

Immunophenotyping. Due to the high concordance between spleen and lymph node, immunophenotyping results are depicted for the spleen. Immunophenotyping of tumor cells in peripheral blood, spleen, and lymph node of Eµ-MTCPI and Eµ-TCL1 mice by flow cytometry was performed as follows: APC rat anti-mouse CD45 (1/100 dilution; Biosciences Cat #559864), FITC rat anti-mouse CD45R/B220 (1/100 dilution; Biosciences Cat #553023). Immunomodulatory assessment of Eµ-MTCPI peripheral blood by flow cytometry was performed as follows: PE hamster anti-mouse CTLA4 (1/16 dilution; Biosciences Cat #535720), PE hamster IgG1 κ isocontrol type (1/16 dilution; Biosciences Cat #553972), BV421 rat anti-mouse CD5 (1/385 dilution; Biosciences Cat #562739), FITC rat anti-mouse CD19 (1/200 dilution; Biosciences Cat #564509). CD5+, CD19+ lymphocytes effacing the bone marrow and lymph nodes in both ibrutinib and vehicle treated mice, while infiltrates are less severe in the liver and spleen of mice treated with ibrutinib. Neoplastic cells are observed as discrete, basophilic nodules in the livers (arrows) and spleens (circled regions) of the ibrutinib-treated mice, whereas in the vehicle-treated mice, neoplastic cells tended to form diffuse sheets or larger/condensed nodules. All organs visualized at 10x, scale bars are 200 μm.

RNA-sequencing. Cell pellets were captured and washed in PBS on ice prior to resuspension in TRIzol reagent and stored at −80°C. Total RNA was isolated from TRIzol suspensions using a chloroform/ethanol extraction method and quantified via Qubit RNA HS Assay kit (Invitrogen). The Clontech SMARTer v4 kit (Takara Bio USA, Inc.) was used for global preamplification. Illumina sequencing libraries were derived from the resultant cDNA using the Illumina Nexera XT DNA Library Prep Kit following manufacturer’s instructions. RNA-sequencing libraries were prepared with the Illumina Tru-Seq stranded kit and sequenced on a HiSeq 4000 targeting 40 × 10^6 fragments per sample. Transcript-level abundances were estimated using Salmon69, with the genome mouse release 23, imported using tximport64, with normalization and differential expression computed with DESeq265. Data processing was performed according to the CLEAR workflow38, which identifies reliably quantifiable transcripts in low-input RNA-seq for differentially expressed gene (DEG) transcripts using gene coverage profiles. MiXCR (v3.0.5β)29 was used with default parameters except the RNA-seq alignment was replaced with kalign62 to identify preprocessed reads containing CDR3 regions from B-cell heavy, kappa, and lambda chains, generating a list of unique CDR3 sequences associated with their relative abundances and specific V(D)J gene usage. MiXCR then generates a list of unique CDR3 sequences associated with their relative abundances and specific V(D)J gene usage. To verify expression of the human MTCPI and TCL1 transgene (hMTCPI & hTCL1) in mice, transcript level abundances were estimated using Salmon with a modified genome mouse reference that contained sequences from human MTCPI and human TCL1 genes extracted from the gecg38 human reference.

Therapeutic dosing in Eµ-MTCPI mice. Eµ-MTCPI littermate mice were randomized and enrolled to receive continuous ibrutinib (~30 mg/kg/day via drinking water) or vehicle administration beginning at 2 months of age orally via supplemented drinking water. Mice were followed for leukemia onset and overall survival until reaching predefined removal criteria.

Adaptive transfer studies were conducted using 1×10^6 viable Eµ-MTCPI splenocytes injected via tail vein to immune competent C57BL/6NTac mice. Engrafted mice were monitored for leukemic expansion via weekly flow cytometry of peripheral blood collected by cheek punch. Upon reaching >20% CD5+/CD19+/-CD45+ cells in peripheral blood mice were randomized and enrolled to receive either ibrutinib (25 mg/kg daily oral gavage (o.g.)) or vehicle (0.5% methylcellulose/1% Tween80 o.g.). Continued leukemia progression was monitored by weekly flow cytometry analysis of peripheral blood until reaching predefined removal criteria.

Statistics. Unless otherwise noted, analyses were performed by independent statisticians within the OSU Center for Biostatistics according to methods from previously described models. All analyses were performed using SAS/STAT software, version 9.4 (SAS Institute, Inc., Cary, NC). Evaluation of the difference in mean gene expression across cell types collected from the Blueprint DCC gene expression portal generated a two-tailed p value using an unpaired t test with Welch’s correction. For patient data, associations between MTCPI expression grouped by quartile and demographic, clinical, and molecular features were assessed using Fisher’s exact and Kruskal–Wallis tests. MTCPI expression was correlated with PFS using a Cox stratified proportional hazards model, stratified on study cohort. Further modeling included additional important demographic and molecular variables. Multiple imputation estimated missing data and combined results for 20 datasets64. All p values were two-sided and p < 0.05 were considered statistically significant.
significant. No control for multiple comparisons was made. The correlation between MTCP1 expression and PFS was visualized using Kaplan–Meier plots, grouping patients into quartiles according to MTCP1 expression. For mouse survival experiments, survival curve estimates for both overall survival and time to disease onset were calculated using the Kaplan–Meier method and differences in curves were initially assessed using the log-rank test. Next, hazard ratios (HR) and 95% CI were obtained from Cox proportional hazards models to evaluate differences between founder lines/mtTCL1 mice or treatment groups. Mixed effects models were used to assess changes in disease burden over time. Where applicable, data were log-transformed to reduce skewness.

**Illustrations.** Artistic renderings were created and exported under a paid subscription with Biorend.com. Biologic assembly of the proposed 3-dimensional structure of MTCP1 and TCLI proteins, as determined by x-ray diffraction protein crystallography, was visualized and exported under a University supported subscription using the PYMOL Molecular Graphics System, Version 2.3.5. Unless otherwise noted, data were visualized using GraphPad Prism version 8.3.1 for Windows, GraphPad Software, San Diego, California USA.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The RNA-sequencing data generated in this study have been deposited in the GEO database under accession code #GSE176094. The MTCP1 expression and CLL patient outcomes data, from CALGB studies “10101” and “9712” used in this study, are not publicly available but can be accessed from the authors of these studies (CALGB “9712”. NCT00003248; CALGB “10101” – NCT00009860)22-26. All other source data are provided as a Supplementary File. Source data are provided with this paper.

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Author contributions

J.S.W. and Z.A.H. designed and conducted experiments, generated data and figures, analyzed data, interpreted results, and wrote the paper. S.S., J.C., K.W., J.N.S., C.B.C., C.T.G., A.P., M.Y., and F.P. conducted experiments and generated figures. I.C. and R.H. prepared and interpreted histopathology data. L.P.B. and B.R.W. performed animal experiments. Z.A.H., K.W., V.C., J.S.B., and R.L. contributed to the generation of the mouse model. J.M.L. and N.A.H. conducted and interpreted FISH analysis. K.M. and J.A.W. provided and interpreted CLL patient data. A.S.R., A.M.L., and H.G.O. conducted statistical analysis. J.S.B., J.C.B., and R.L. planned the project, acquired funding, supervised the study, interpreted results, and reviewed the paper. These senior authors (J.S.B., J.C.B., R.L.) contributed equally. All authors read and approved the final version of the paper.

Competing interests

J.S.B. has performed consulting for AbbVie, AstraZeneca, Innate, and KITE Pharma. J.C.B. performed consulting for AstraZeneca, Takada, Novartis, Pharmacyclics, Syndex, and Trillium. J.C.B. chairs the scientific board of Vincera Pharmaceuticals and has significant equity in this company. All other authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Rosa Llapalomba.

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