NIPA (Nuclear Interaction Partner of ALK) Is Crucial for Effective NPM-ALK Mediated Lymphomagenesis

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The NPM-ALK fusion kinase is expressed in 60% of systemic anaplastic large-cell lymphomas (ALCL). A Nuclear Interaction Partner of ALK (NIPA) was identified as a binding partner of NPM-ALK. To identify the precise role of NIPA for NPM-ALK-driven lymphomagenesis, we investigated various NPM-ALK+ cell lines and mouse models. Nipa deletion in primary mouse embryonic fibroblasts resulted in reduced transformation ability and colony formation upon NPM-ALK expression. Downregulating NIPA in murine NPM-ALK+ Ba/F3 and human ALCL cells decreased their proliferation ability and demonstrated synergistic effects of ALK inhibition and NIPA knockdown. Comprehensive in vivo analyses using short- and long-latency transplantation mouse models with NPM-ALK+ bone marrow (BM) revealed that Nipa deletion inhibited NPM-ALK-induced tumorigenesis with prolonged survival and reduced spleen colonies. To avoid off-target effects, we combined Nipa deletion and NPM-ALK expression exclusively in T cells using a lineage-restricted murine ALCL-like model resembling human disease: control mice died from neoplastic T-cell infiltration, whereas mice transplanted with Lck-CreTg/wtNipafox/fox NPM-ALK+ BM showed significantly prolonged survival. Immunophenotypic analyses indicated a characteristic ALCL-like phenotype in all recipients but revealed fewer “stem-cell-like” features of Nipa-deficient lymphomas compared to controls. Our results identify NIPA as a crucial player in effective NPM-ALK-driven ALCL-like disease in clinically relevant murine and cell-based models.

Keywords: NIPA, NPM-ALK, anaplastic large cell lymphoma, lymphomagenesis, transplantation mouse model
INTRODUCTION

Anaplastic large cell lymphoma (ALCL) is an aggressive peripheral T-cell non-Hodgkin’s lymphoma, usually presenting at advanced stages as a systemic disease with multi-nodal involvement. It is characterized by anaplastic morphology, expression of CD30 (Ki-1), and a cohesive growth pattern with infiltration of lymph node sinuses (1, 2). Typical T-cell markers are rarely detectable in ALCL; about 40% have a so-called null-cell phenotype as they lack both T-cell marker and receptor rearrangements (2–4). A t(2;5)(p23;q35) translocation leads to the expression of the chimeric Nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) in approximately 60% of systemic ALCL cases (5). Through NPM-mediated homodimerization, ALK is constitutively activated in ALCL, leading to increased proliferation and tumorigenesis through promitotogenic, antiapoptotic, and transforming pathways, particularly STAT3, JUNB, AP-1, MAPKs, and PI3K/mTOR/AKT (6–12). Various animal models have demonstrated the essential role of NPM-ALK in ALCL pathogenesis (13–18), yet the key pathway for lymphomagenesis remains to be identified.

ALK+ ALCL is diagnosed mainly in children and young adults, whereas the ALK-negative form is more common in older adults (19, 20). Although initial responses to standard chemotherapy are given the young age of onset. Studies with ALK-inhibitors and relapse within years or later, which is particularly relevant for physiologic mitotic timing and DNA damage repair, its absence bears potential for both malignant transformation and apoptosis.

MATERIALS AND METHODS

Constructs, Cell Culture, and Virus Generation

For virus production, Phoenix E ecotropic packaging cells (a kind gift from G. Nolan, Stanford, CA) were transiently transfected with the retroviral construct MSCV-STOP-NPM-ALK-IRESCFP (MSNAIE), Mig-NPM-ALK+, pBABE-puroRNIPA, and viral supernatants were collected as described previously (14, 17, 18, 37). Retroviral titers were determined by the transduction of NIH/3T3 cells (DSMZ) as described previously (17).

MTS Assay and Soft Agar Assay

The assays were performed as previously described (36). In brief, retrovirally infected Ba/F3 or Karpas299 cells express NPM-ALK and miR-Nipa [as previously described (38)] or miR-Nipa (5’TGC TGT TGA CAG TGA GCG CTC CAT TGGAAT TCA CAA GCA ATA GTG AAG CCA CAG ATG TAT TGTTG ATG ATTCCA ATG GAA TGATAG CTA CCG CCT CGG A-3’) were plated on 96-well plates in triplicates (5,000 cells in 100 µl of RPMI). To assess cell proliferation, MTS reagent (Promega, Madison, WI, USA) was added to the cells at the indicated time points and incubated for 2 h at 37°C. Extinction at 492 nm was measured using a microplate reader (Tecan, Männedorf, Switzerland).

For soft agar proliferation assays, we prepared primary Nipa+ or Nipa− MEFs from embryos (E13.5) and cultured them in DMEM (PAA Laboratories) supplemented with 15% FCS under low oxygen conditions. Early passages only were used for the indicated experiments. Nipa+/− MEFs were retrovirally infected with vectors containing NPM-ALK and Flag-NIPA wt. The assay was performed as previously described (39). A total of 25,000 and 100,000 cells were plated in soft agar in 6-well plates. Colonies were counted between days 15 and 20 after plating.

EdU Cell Cycle Assay

For cell cycle measurements, EdU along with FxCycleViolet, was used according to the instructions of the manufacturer.
Immunoblot
Immunoblotting was performed as described previously (33, 34). Antibodies against βACTIN (A5316) and NIPA (ZC3HC1, HPA024023) were purchased from Sigma. ALK (cs-3333) was purchased from Cell Signaling, GAPDH (OSG-00033G) from Osenses. Quantification of immunoblots was performed using LabImage 1D L340 software (Intas Science Imaging, Gottingen, Germany).

Reagents
Recombinant murine Interleukin-3 (IL-3), IL-6, and SCF were purchased from R&D Systems (Minneapolis, MN, USA). Fetal calf serum, 5-Fluorouracil, and Polybrene were purchased from Sigma-Aldrich (St. Louis, MO, USA). DMEM (Dulbecco’s Modified Eagle Medium) and ES cell FBS (fetal bovine serum) were purchased from Thermo Scientific (Waltham, USA). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA, USA). TAE-684 was purchased from Axon Medchem (Groningen, NL).

Mice
The Lck-Cre mouse line (B6.Cg-Tg(Lck-cre)548Jxm/fj) was obtained from the Jackson Laboratory (Bar Harbor, Maine, USA). Nipa<sup>lox/lox</sup> mice were generated using a conditional knockout strategy (34). To achieve tissue-specific Nipa deletion, we crossed Nipa<sup>lox/lox</sup> mice with Lck-Cre transgenic mice. Littermates or age- and sex-matched wild-type mice were used as controls. All mice were backcrossed to a C57BL/6 background for more than ten generations. The animals were housed in a special cage system with autoclaved food and acidified water at the University of Freiburg. All procedures were performed in accordance with national and institutional guidelines for animal care and experiments.

Transplantation Assays
Murine bone marrow was collected from Lck-Cre wildtype Nipa<sup>ko/ko</sup> and Nipa<sup>wt/wt</sup> or Lck transgenic Nipa<sup>lox/lox</sup> and Nipa<sup>wt/wt</sup> mice and infected as described previously (14, 38, 40). Briefly, 12-week-old male donor mice were treated once with 5-Fluorouracil (150 mg/kg) on day - 4 and BM cells were harvested from the tibia and femur. After preincubation overnight in BM media (DMEM, 30% FBS, 10 ng/ml mIL-3, 10 ng/ml mIL-6, and 50 ng/ml mSCF), BM cells were infected with retroviral supernatant as described previously (41). The infection efficiency was determined by flow cytometric analysis of GEF expression. Female C57BL/6 wild-type recipient mice were irradiated with 850 rad and transplanted with the indicated cells via the tail vein injection. Peripheral blood was taken at the indicated time points and WBCs were measured using an automated counter (ABC scil vet). Transplanted mice were monitored for signs of disease and sacrificed and analyzed based on clinical signs.

Flow Cytometry Analysis
Flow cytometry analysis was performed as described (35). The BD LSR Fortessa (BD Biosciences, Heidelberg, Germany) was used for analysis. Antibodies used to stain cell surface proteins were anti-mouse CD4 (GK1.5), CD8a (53-6.7), CD11b (Mac-1, M1/70), CD25 (PC61.5), CD44 (IM7), CD45R/B220 (RA3-6B2), CD45 (30-F11), CD90.2 (THY1.2, 53-2.1), CD117 (c-KIT, 2B8), CD127 (IL-7Rα, A7R34), GR1 (Ly-6G, RB6-8C5), SCA1 (D7), and TER119 (TER119) and the corresponding isotypes, which were obtained from BD Biosciences or eBiosciences (Frankfurt am Main, Germany).

Statistical Analysis
A two-sided Student’s t-test was used for statistical analyses. The mean ± standard deviation was analyzed as indicated. The survival curves were produced using a log-rank (Mantel–Cox) test. P-values were defined as *p <0.05, **p <0.01, ***p <0.001, and ****p <0.0001.

RESULTS
NIPA Deficiency Impairs NPM-ALK Mediated Colony Formation and Viability In Vitro
To analyze the impact of NIPA on NPM-ALK mediated transformation and colony formation, we performed in vitro soft agar assays. Nipa<sup>ko/ko</sup> mouse embryonic fibroblasts (MEFs) were retrovirally infected with NPM-ALK and NIPA or empty vector control. As seen in Figure 1A, Nipa-deficient MEFs infected with NPM-ALK showed significantly lower numbers and smaller sizes of colonies in soft agar assays than NIPA-re-expressing controls (28.9 colony forming units (CFUs) vs. 58.8 CFUs; p = 0.008). Western blotting revealed the correct expression of NPM-ALK and NIPA (Figure 1B). In the absence of NPM-ALK, no colony growth was observed in either group, suggesting that Nipa deficiency alone has no transformative potential in MEFs in soft agar assays.

Using targeted genetic approaches, an efficient and durable NIPA knockdown of more than 70% was achieved in the murine IL-3 dependent Ba/F3 cell line and the human ALCL cell line Karpas299 (Figure 1C). Upon efficient NIPA downregulation, Ba/F3 cells were retrovirally infected with NPM-ALK. Proliferation was assessed by MTS assays under IL-3 withdrawal, where the optical density (OD) reflects the metabolism of MTS reagent and thus the number of viable cells present. NIPA knockdown significantly impaired the proliferation of NPM-ALK-positive Ba/F3 and Karpas299 cells. Within 24 h, the number of viable NPM-ALK-positive Ba/F3 grew 4.2 fold, whereas only 3.1 fold in NIPA knockdown cells (Figure 1D). Two-dimensional cell cycle analyses revealed no differences in the cell cycle profile of NPM-ALK-positive cells upon NIPA knockdown, pointing to a cell-cycle-independent function of NIPA in dependence of NPM-ALK (Supplementary Figures 1A, B).

To expand our data to human cells, we designed NIPA siRNAs targeting the human NIPA mRNA and downregulated NIPA in the human ALCL cell line Karpas299. Effective NIPA downregulation resulted in a significantly reduced proliferation
(75% compared to controls) of ALCL cells measured at numerous time points after seeding (Figure 1E). Interestingly, NIPA downregulation in Karpas299 cells showed a significantly higher susceptibility to the ALK inhibitor TAE-684 (Figure 1F), suggesting a possible synergistic effect of ALK inhibition and NIPA knockdown.

Loss of NIPA Prolongs Survival in Short and Long Latency NPM-ALK Driven Murine Tumorigenesis

To analyze the impact of NIPA on NPM-ALK-induced tumorigenesis, we used a retroviral murine BM transplantation model for NPM-ALK-driven malignancies. As previously
demonstrated by Miething et al. (17), transplantation of NPM-ALK-positive bone marrow cells (BMCs) in lethally irradiated recipient mice leads to two distinct phenotypes (polyclonal histiocytic malignancy vs. monoclonal B-lymphoid tumors), depending on disease latency. We performed analogous transplantation experiments using Nipa\(^{\text{ko/ko}}\) and Nipa\(^{\text{wt/wt}}\) donor BMCs. For the short latency model, mice were given 300,000 BMCs with 3.0% NPM-ALK (EGFP) positive cells. For the long latency model, 200,000 cells with 0.4% NPM-ALK (EGFP) positive cells were injected. Independent of the model used, mice transplanted with Mig NPM-ALK\(^{Nipa^{\text{ko/ko}}\text{BMCs}}\) showed significantly prolonged survival with 28.5 vs. 27 days (p = 0.03) in short latency and 118 vs. 84 days (p = 0.008) in the long latency model, respectively (Figures 2A, B).

In the short latency model, the progression of the disease was furthermore assessed by the number of spleen colonies. Animals transplanted with Mig NPM-ALK\(^{Nipa^{\text{ko/ko}}\text{BMCs}}\) bone marrow were found to have a significantly lower number of spleen colonies than controls, with 10 colonies per spleen vs. 28 in controls (p <0.001) (Figure 2C). However, the disease immunophenotype was not altered by the absence of NIPA in both the long and short latency models (data not shown). Taken together, our results highlight the crucial role of NIPA in NPM-ALK-driven tumorigenesis.

Deletion of Nipa Delays Lymphoma Progression in an ALCL-Like Mouse Model

Based on these results, we hypothesized that NIPA influences NPM-ALK-driven transformation in an ALCL mouse model resembling the human clinical phenotype. This ALCL-like model is based on a lineage-specific Cre/LoxP-dependent expression of NPM-ALK by the retroviral construct MSCV\(^{-}\)STOP\(\)NPM-ALK\(\)IRES\(\)EGFP (MSNAIE) (Supplementary Figure 2A). Infection of Lck\(^{\text{TG/wt}}\) BMCs with MSNAIE retrovirus and transplantation into lethally irradiated wild-type recipient mice leads to a systemic CD30-positive ALCL-like T-cell lymphoma (14, 18).

To establish a Nipa-deficient ALCL-like disease without “off-target” effects of Nipa deficiency, we used donor BMCs from Lck\(^{\text{TG/wt}}\)\(\text{Nipa}^{\text{fl/ox}}\) mice for MSNAIE infection, thus restricting NPM-ALK expression and Nipa deletion to the identical T cells. Transplanted mice were monitored for clinical signs of disease, such as wasting, tachydyssnea, lymphadenopathy, and changes in the complete blood count. Lck\(^{\text{TG/wt}}\)\(\text{Nipa}^{\text{fl/ox}}\) MSNAIE transplanted mice showed a later onset of disease with significantly prolonged survival. The median survival was significantly shorter (p = 0.002) with 121 days in Lck\(^{\text{TG/wt}}\)\(\text{Nipa}^{\text{wt/wt}}\) MSNAIE transplanted mice compared to 143 days in Lck\(^{\text{TG/wt}}\)\(\text{Nipa}^{\text{fl/ox}}\) (Figure 3A). At the final stage of the

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**FIGURE 2** | Loss of NIPA prolongs survival in short and long latency NPM-ALK driven murine tumorigenesis. (A) Kaplan–Meier survival curve of mice transplanted with 300,000 Nipa\(^{\text{wt/wt}}\) and Nipa\(^{\text{fl/ox}}\) bone marrow cells infected with Mig NPM-ALK\(^{3\%}\) (3%). Median survival was 28.5 days (Nipa\(^{\text{wt/wt}}\), n = 8) vs. 27 days (Nipa\(^{\text{fl/ox}}\), n = 8). (B) Kaplan–Meier survival curve of mice transplanted with 200,000 Nipa\(^{\text{wt/wt}}\) and Nipa\(^{\text{fl/ox}}\) bone marrow cells infected with Mig NPM-ALK\(^{0.4\%}\) (0.4%). Median survival was 118 days (Nipa\(^{\text{wt/wt}}\), n = 12) vs. 84 days (Nipa\(^{\text{fl/ox}}\), n = 12). (C) Number of spleen colonies in mice transplanted with Nipa\(^{\text{wt/wt}}\) and Nipa\(^{\text{fl/ox}}\) BMCs infected with Mig NPM-ALK\(^{3\%}\) at final stage of disease. Mean amount of colonies per spleen were 10 (Nipa\(^{\text{wt/wt}}\), n = 5) vs. 28 (Nipa\(^{\text{fl/ox}}\), n = 5). Representative spleens shown. *p < 0.05, **p < 0.01, ***p < 0.001.
distribution in both Nipa subpopulation analyses revealed a heterogeneous CD4/CD8 (Kreutmair et al., 2020a; Shoumariyeh et al., 2020). CD4/CD8 further referred to as (Figures 2C, D). Correct Nipa deletion in lymphoma cells was validated by PCR analysis (Figure 3C) and lymphomas of LckCreTg/wtNipafox/fox MSNAIE transplanted recipients were further referred to as Nipako/ko. As has previously been reported for wild-type lymphomas, immunophenotyping of Nipa ko/ko lymphoma cells showed a pure T-cell phenotype with negativity for myeloid and B-cell markers in the thymus, spleen, lymph nodes, peripheral blood, and bone marrow (Figure 3D) (Kreutmair et al., 2020a; Shoumariyeh et al., 2020). CD4/CD8 subpopulation analyses revealed a heterogeneous CD4/CD8 distribution in both Nipa−/− and Nipa+/+ lymphomas (Figure 3E), which was, despite minor variations, similar in both groups with distinct CD4/CD8-double positive, CD4/CD8-double negative, and CD4- and CD8-single positive populations. Further analysis regarding the DN stages showed no significant difference between Nipa+/+ and Nipa−/− lymphomas (Figure 3F). Our results therefore show that NIPA seems to play a significant role in NPM-ALK-induced lymphogenesis but does not alter the disease immunophenotype of ALCL.

DISCUSSION

NIPA has recently been described as a crucial regulator of mitotic entry and bone marrow failure (35, 42), but has not yet been analyzed in NPM-ALK-induced lymphomas, where it was initially found as an interaction partner of NPM-ALK. Ouyang et al. have shown that NIPA interacts with NPM-ALK in a kinase-dependent manner and can protect Ba/F3 cells from apoptosis (31). The results of this study identify NIPA as a relevant player in efficient NPM-ALK mediated lymphogenesis. In our study, in vitro assays with different cell lines showed that proliferation and transformation of NPM-ALK-positive MEFs, Ba/F3, and Karpas299 cells were significantly impaired upon NIPA deficiency or downregulation, which was not due to the already described cell cycle-dependent function of NIPA. In vivo experiments in different murine NPM-ALK-driven tumor models extended these results and demonstrated that transplantation of NPM-ALK-positive Nipa ko/ko BMCs and BM T-lineage restriction of Nipa deletion and NPM-ALK expression led to significantly prolonged survival compared to Nipa+/+ transplanted animals. The absence of immunophenotype changes may reflect the known functions of the protein as a regulator of the cell cycle and DNA damage repair rather than cell differentiation.

Based on the prolonged survival of recipient mice transplanted with Nipa-depleted cells in three different BM transplantation models and regular oncogenic signaling pathways independent of NIPA, we hypothesized that NIPA is relevant for ALCI lymphoma initiation. Recent studies of Nipa−/− mice demonstrated reduced numbers and function of hematopoietic stem cells (HSCs). Nipa-deficient HSCs showed cell-intrinsic defects leading to reduced proliferation capacity, accumulation of DNA damage, and cell death due to impaired DNA damage/FA/BRCA pathway. Furthermore, aged or replication-stressed Nipa-deficient animals developed bone marrow aplasia (35). We found a lymphoma subpopulation characterized by the stem cell markers LineagecKIT+cSCA1− being significantly reduced in Nipa ko/ko lymphomas. This may influence the observed prolonged disease latency by regulating the lymphoma stem cell reserve. In the past years, the so-called “cancer stem cell theory” has been described for various hematological and solid malignancies. According to this theory, only a small percentage of cells in an overall heterogeneous malignancy show tumor promoting characteristics, such as specific surface markers, gene expression profiles, or the ability to generate identical xenografts (14, 43, 44). Various studies have explored the originating cells of NPM-ALK-positive ALCI, and there is accumulating evidence that lymphoma initiation starts in a primitive cell population at an undifferentiated T cell or even HSC-like level—genetically reprogrammed and independent of their phenotype (14). Moti et al. have identified a side population in ALCI that proliferated more than the main population and could therefore give rise to xenografts (45). This side population expressed a gene profile similar to that of early thymic progenitor cells, supporting the hypothesis of a stem cell origin. Yet regarding the immunophenotype, the ability to form xenografts was independent from the presence of hematopoietic stem cell markers (45). A different study showed the importance of the embryonic stem cell factor Sox2 for ALCI xenograft growth, a hint towards the importance of progenitor cells for tumor propagation (46).
In the case of Nipa deficiency, the number of both healthy hematopoietic stem cells in aged mice and stemness marker-expressing lymphoma cells in the NPM-ALK-positive disease is reduced. In healthy HSCs, NIPA plays a crucial role in DNA damage repair as a regulator of FANCD2, the key player in the Fanconi anemia pathway, rather than regulating the cell cycle.

**FIGURE 3** | Deletion of Nipa delays lymphoma progression in an ALCL-like mouse model. (A) Kaplan–Meier survival curve of mice transplanted with LckCreTg/wtNipawt/wt MSNAIE and LckCreTg/wtNipaflox/flox MSNAIE bone marrow. Median survival was 121 days (Nipa+/*, n = 9) versus 143 days (Nipa−/−, n = 11). Data from three independent transplantations was analyzed. (B) Representative images of infiltrated organs from mice transplanted with LckCreTg/wtNipaflox/flox MSNAIE BMCs. LN, lymph node; T, thymus; S, spleen. (C) Gel electrophoresis showing lymphoma genotype of representative LckCreTg/wtNipa+/* and LckCreTg/wtNipa−/− MSNAIE transplanted mice in different lymphatic organs, correlated to EGFP-positivity and expression of T-cell markers. (D) Immunophenotyping of representative thymic lymphoma tissue determined by flow cytometry. (E) Mature T-cell distribution determined by flow cytometry for CD4 and CD8 in EGFP+ thymic cells of LckCreTg/wtNipa+/* (n = 9) and LckCreTg/wtNipa−/− (n = 16) transplanted mice. (F) DN T-cell subpopulations determined by flow cytometry due to CD44 and CD25 expression in EGFP+ thymic cells of LckCreTg/wtNipa+/* (n = 8) and LckCreTg/wtNipa−/− (n = 14) transplanted mice. Representative flow cytometry gating strategy on the right. *p < 0.05, **p < 0.01, ***p < 0.001. Data shown as mean ±SD.
itself. Thus, replication stress is a major risk factor for the Nipa-deficient stem cell pool. Taking into consideration that cancer stem cells necessarily undergo substantial replication stress at the time of lymphoma initiation and development, one may hypothesize that NIPA regulates this phase of lymphatic disease. Thus, NIPA may act at the level of the ALCL "stemness" cell population, but it might as well be that it positively influences the malignant transformation in general by protecting cells from apoptosis. However, in vitro analysis of MigNPM-ALK transduced Ba/F3 and Karpas299 cells transfected with either pLMPmiRNAp or pLMPmiRctrl did not demonstrate major differences in apoptosis (data not shown).

It would be interesting to see if a phosphorylation-deficient mutant of Nipa at the recently identified serin/threonin sites relevant for NIPA-NPM-ALK binding changed the course of the ALCL-like disease. Taking into account that a phosphorylation-deficient Nipa has negatively influenced proliferation upon NPM-ALK expression in vitro (36), it is possible that those sites also play a crucial role in vivo. One could furthermore hypothesize that this phosphorylation-deficient Nipa mutant also led to impaired DNA damage/FA/BRCA pathway or deregulation of mitotic entry in NPM-ALK-driven lymphomagenesis. Further characterization of the NIPA/NPM-ALK interaction in in vivo mouse models and human ALCL might be necessary to elucidate the exact underlying mechanistic pathways.

In summary, we could show that NIPA is essential for effective initiation of NPM-ALK-driven ALCL-like disease in a clinically relevant mouse model, while it seems dispensable for the lymphoma immunophenotype. Highlighting the importance of the NIPA/NPM-ALK axis in lymphoma development, clinical assessment of NIPA may provide a basis for future therapeutic approaches.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The animal study was reviewed and approved by the Regierungspräsidium Freiburg, 79095 Freiburg im Breisgau.
AUTHOR CONTRIBUTIONS

Conceptualization, JD and ALI. Methodology, SK, CK, CA-L, CM, and ALI. Investigation, SK (Figures 3, 4), LJL (Figures 1, 3), CK (Figure 3), CA-L (Figure 2), VS (Figure 3), and TM (Figure 3). Data curation, SK (Figures 3 and 4), LJL (Figures 1 and 3), CK (Figure 3), CA-L (Figure 2), and VS (Figure 3). Formal analysis, SK (Figures 3, 4), LJL (Figures 1, 3), CK (Figure 3), and CA-L (Figure 2). Resources, ALI. Writing—original draft preparation, SK and LJL. Writing—review and editing, SK, LJL, CK, CA-L, SY, VS, TM, AM-R, CY, SPG, CM, JD, and ALI. Visualization, SK and LJL. Supervision, SK, CK, and ALI. Project administration, SK, CK, and ALI. Funding acquisition, ALI and JD. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.875117/full#supplementary-material

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