Targeted therapy in JMML: where are we now?

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Abstract

Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive clonal neoplasm of early childhood, classified as an overlap myeloproliferative/myelodysplastic neoplasm by the World Health Organization (WHO). In ninety percent of the patients with JMML typical initiating mutations in the canonical Ras pathway genes *NF1*, *PTPN11*, *NRAS*, *KRAS* and *CBL* can be identified. Hematopoietic stem cell transplantation (HSCT) currently is the established standard of care in most patients, although long-term survival is still only 50-60%. Given the limited therapeutic options and the important morbidity and mortality associated with HSCT, new therapeutic approaches are urgently needed. Hyperactivation of the Ras pathway as disease mechanism in JMML lends itself to the use of targeted therapy. Targeted therapy could play an important role in the future treatment of patients with JMML. This review presents a comprehensive overview of targeted therapies already developed and evaluated *in vitro* and *in vivo* in patients with JMML.

Abbreviations

JMML	Juvenile myelomonocytic leukemia
WHO	World Health Organization
HSCT	Hematopoietic stem cell transplantation
HSC	Hematopoietic stem cell
BM	Bone marrow
OS	Overall survival
HMA	Hypomethylating agents
GM-CSF	Granulocyte-macrophage colony-stimulating factor
iPSCs	Induced pluripotent stem cells
FTIs	Farnesyltransferase inhibitors
EFS	Event-free survival
T-ALL	T-cell acute lymphoblastic leukemia
GVHD	Graft versus host disease
CAR	Chimeric antigen receptor
CLL	Chronic lymphocytic leukemia
CMML	Chronic myelomonocytic leukemia
NF1	Neurofibromatosis type 1
CML	Chronic myelogenous leukemia

Introduction

JMML characteristics and subtypes

JMML is a rare and aggressive clonal neoplasm of early childhood. The clonal growth of an abnormal multipotent hematopoietic stem cell (HSC) leads to the characteristic proliferative features, such as monocytosis, splenomegaly and also a moderately elevated percentage of myeloblasts. In contrast, erythropoiesis and thrombopoiesis are typically decreased due to dysplastic changes in the bone marrow (BM) (1-4).

Although progress has been made in elucidating the pathogenesis of the disease, hematopoietic stem cell transplantation (HSCT) currently is the established standard of care, and results in long-term overall survival (OS) of only 50-60% of patients (5-7). These outcomes have not changed substantially over the last several decades. Despite the intensity of this treatment, relapse of JMML is the most common cause of death. To date, no other treatment options have been able to alter the natural course of this disease.

Research in the past decade was mainly focused on the identification of primary and secondary mutations (8-10), alterations in the non-coding transcriptome (microRNAs, lncRNAs, circRNAs) (11-14), and aberrant genomic DNA methylation (15-18). The latter has identified a subgroup of JMML patients with high DNA methylation and very poor prognosis (17, 18). The identification of abberant methylation patterns in patients with JMML resulted in the use of hypomethylating agents (HMA), such as azacitidine, as bridge to HSCT (19). Although this treatment had no significant effect on DNA methylation between responders and non-responders, evidence shows that treatment with azacitidine may contribute to a better pre-transplant disease state and thus a better outcome of HSCT and a longer overall survival (19-22). This observation resulted recently in FDA approval for azacitidine in newly diagnosed JMML.

In approximately 90-95% of patients with JMML, canonical mutations in the *NRAS* and *KRAS* (20%-25%), *PTPN11* (35%), *NF1* (10%-15%) or *CBL* (10%-15%) genes are observed (23, 24), strongly linking the disorder to hyperactivation of the RAS/MAPK pathway. This characteristic makes this disease attractive for targeted therapies against different components of this pathway. Although initial expectations of targeted therapy in JMML were high, major hurdles were encountered. For instance, Ras is an important component of normal cell physiology, and targeted therapy can cause considerable systemic toxicity or even development of other malignancies. Nevertheless, promising steps have been taken in the last decade (25).

Given the limited therapeutic options and their associated morbidity, new therapeutic approaches are urgently needed. Based on recent insights into disease pathogenesis, there is a renewed interest in evaluating approaches directly targeting the RAS pathway. Here, we provide a comprehensive overview of targeted therapies already developed and tested in JMML, their targets and their efficacy *in vitro* and *in vivo* in patients with JMML.

Novel targeted appoaches in JMML

The rate of somatic events in JMML is much lower compared to other cancers (0.38 versus 0.61 events/megabase [Mb]/case on average in childhood cancer) (26-28), strenghtening the hypothesis that hyperactivation of the Ras pathway alone is sufficient for disease propagation. This relative genetic simplicity, combined with RAS-pathway mutations as a "first hit" and mostly linear patterns of clonal evolution makes this disease suitable for pharmacological inhibition of the Ras pathway. In the following section, we provide an up-to-date overview of novel Ras targeted therapies with proven *in vitro* or *in vivo* activity in JMML. An overview of different targets, treatment options and evidence is given in FIGURE 1 and TABLE 1.

Therapies targeting GM-CSF signaling pathways

Characteristic for JMML cell proliferation is hypersensitivity of myeloid progenitors to granulocyte-macrophage colony-stimulating factor (GM-CSF), which elicits a cascade of downstream signaling and transcription factor activation. Although in JMML abnormalities of the GM-CSF-receptor haven't been identified, targeting of this pathway is studied using different approaches, including immunotherapies and small molecule inhibitors of downstream components of the GM-SCF receptor signaling pathway (29-33).

The *GM-CSF-analogue* E21R is an antagonist of the GM-CSF-receptor. *In vitro*, use of this antagonist resulted in inhibition of colony growth of JMML cells (32), whereas in a mouse model of JMML it resulted in improved physical condition at the end of the treatment period (33). Use of this compound in a patient, initially resulted in a fast improvement of the clinical condition a few days after the first cycle was given. In contrast, at the beginning of the third cycle monocytes and myeloblasts increased, suggesting refractory disease to the GM-CSF-analogue. One month after the third cycle, the patient died as a consequence of internal bleeding and multiple organ failure (34). As E21R has been taken off the market twenty years ago, this drug is no longer a therapeutic option.

Lenzilumab is an engineered human IgG1κ monoclonal antibody, directly targeting GM-CSF, which has shown promising clinical efficacy in chronic myelomonocytic leukemia (CMML) patients. In a phase 1 clinical trial durable clinical improvement was achieved in 4 out of 15 patients, providing a proof-of-concept that GM-CSF inhibition is a viable therapeutic strategy in CMML (35). However no formal evaluation of lenzilumab in the treatment of JMML has been reported.

GM3 is an oligonucleotide that binds the promotor of the GM-CSF gene resulting in formation of a *DNA triple helix* and reduction of GM-CSF transcription. In vitro, GM3 reduced colony formation of JMML-cells (30) The specific inhibition of TNFα gene expression by a catalytic RNA molecule (ribozyme) also downregulated the expression of GM-CSF in JMML cells and GM-CSF dependent colony formation was reduced (36). Although, no further *in vitro*, animal model or clinical studies have been conducted with these compounds.

JAK targeting

Targeting JAK2, a tyrosine kinase in the GM-CSF pathway is another attractive therapeutic approach in JMML (37).

JAK-inhibitors ruxolitinib and momelotinib were shown to effectively inhibit colony growth in induced pluripotent stem cells (iPSCs) of JMML patients with *CBL* mutations., while ruxolitinib also inhibited colony growth of iPSCs carrying the *KRAS* mutation (17).

Interestingly, ruxolitinib attenuated disease in a mouse model of JMML with *Nf1* mutation. A significantly better survival was observed compared to the placebo group. Side effects of ruxolitinib were aggravation of anemia and trombocytopenia (38). Similarly, treatment with ruxolitinib also resulted in an improvement of clinical condition in a mouse model with *Cbl* mutation (39).

Finally, in a phase 1 clinical study with ruxolitinib, 3 patients with JMML were included. At the end of the first month of treatment, 2 of these patients had stabilized disease. One patient even got 5 cycles of ruxolitinib before disease progression occurred (40).

Although not-significant responses with this drug were observed in JMML patients, it showed good safety and tolerability profiles and promising responses in phase 1 clinical trials in patients with CMML. Results of phase 2 clinical trials in CMML are pending (41). In a phase 3 clinical trial in myelofibrosis momelotinib has been compared to ruxolitinib and proved to be noninferior, but offered less symptom control (42).

SHP2-inhibitors

SHP-2 is a non-receptor protein tyrosine phosphatase encoded by the *PTPN11* gene, which is mutated in 35% of the patients with JMML (43). Although, type-1 SHP-2 inhibitors were initially shown to inhibit colony growth of *Ptpn11* mutated mouse cells, it rapidly became clear that they also target SHP-1, which excerts oposing effects to SHP-2, finally nullifying the effect. Development of more selective type 2 inhibitors try to overcome this issue and currently four type 2 SHP-2 inhibitors (JAB-3068, TNO155, RMC-4630, and RLY-1971) are investigated in clinical trials for solid tumors (44-47). In JMML however, no studies with specific SHP-2 inhibitors are conducted yet.

Therapies targeting the RAS-MAPK pathway

Direct inhibition of RAS

RAS is one of the most frequent mutated oncogenes in cancer (25) and also in JMML it plays a central role in the pathogenesis. Development of direct inhibitors of the Ras pathway has not been successfull and consequentially, RAS got the label of being “undruggable”. The most important reasons for this are the high affinity of RAS for GTP making it unfavorable for GTP-competitive inhibitors and the lack of small molecule binding sites besides the GTP binding pocket (25, 48-50). The small molecule RAS inhibitors that have been developed were not potent and selective enough for clinical use. In recent years, new direct RAS-inhibitors have been identified and are being studied in clinical trials. In an alternative approach, Fell et al. designed mutant specific inhibitors (51). Currently, two mutant selective inhibitors (MRTX849 and AMG510) for *KRAS* Gly12Cys are being investigated in phase 2 and 3 clinical trials for non-small cell lung cancer and colorectal cancer. The KRAS Gly12Cys mutation also occurs in

JMML, but only in approximately 1 in 300 cases. Thus, this allele specific inhibitor will not be relevant in JMML

Alternatively, rigosertib is a small molecule RAS mimeticum that binds various effectors which inhibits further activation of the Ras pathway. Mice with a *Kras* mutation in the hematopoetic component were used as a model of JMML. Treatment of mice carrying the *Kras G12D* mutation with rigosertib resulted in reduction of spleen size and improvement of leukocytosis, although mainly neutrophils decreased and the characteristic monocytosis remained. Interestingly, rigosertib had a positive effect on mice survival (52, 53). However, in a phase 3 clinical trial in patients with myelodysplastic syndrome (MDS) no beneficial effect on survival was noted with rigosertib compared to standard of care. (54).

SOS1 inhibitors

SOS refers to a set of genes encoding guanine nucleotide exchange factors which promote the exchange of Ras-bound GDP by GTP. SOS1 inhibitors might be an interesting therapeutic option in JMML. The SOS1 inhibitor BI1701963 is currently investigated in phase 2 clinical trials in KRAS mutated solid tumors, as monotherapy or in combination with trametinib (55, 56). However, no studies on this compound are available in JMML at the moment.

Inhibition of RAS posttranslational modifications

Post-translational farnesylation of RAS proteins by farnesyltransferase is essential for localization of RAS enzymes to the inner cell membrane. Drugs intervening with this process can cause suppression of the Ras pathway.

Although farnesyltransferase inhibitors (FTIs) L-739,749 and L-744,832 were proven to inhibit colony growth of JMML cells in vitro, predominantly driven through their effect on Hras processing, no responses were observed after administration of this drug to Nf1-deficient mice (57, 58).

In a phase 1 trial evaluating the FTI tipfarnib in refractory leukemia patients at a dose of 300 mg/m^2, no complete or partial response were seen in any patient, but one child with JMML remained stable for 6 cycles of tipifarnib (59).

A phase 2 and 3 study investigated the effect of tipifarnib in JMML-patients. In 73% of 47 patients a limited response was observed with a decrease in leukocytosis count and a reduction of spleen and liver size. Unfortunately, no effect on event-free survival (EFS) was demonstrated. The 5-year survival was higher in patients who did not receive tipifarnib in comparison with patients who did (71% vs. 48%, p= 0,06). Few patients experienced disease progression while on tipifarnib, but in conclusion it has no effect on relapse rate or overall survival in JMML (60). Consequently, the use of tipifarnib has been abandoned in hematologic malignancies.

Zoledronate is a third generation bisphosphonate which has the ability to inhibit both farnesyltransferase and geranylgeranyltransferase. In an *in vitro* experiment zoledronate could inhibit colony formation of JMML-cells, but at higher concentrations than in vivo tolerated (61). In a case report, a patient with relapsed JMML was treated with zoledronate after second HSCT. No remarkable effect of this compound was

observed and the patient died 18 days after the first zoledronate infusion due to rapidly progressive disease (62).

The palmitoylation/depalmitoylation cycle is another posttranslational modification that is important for NRAS, HRAS and subtype KRAS4A for a correct localization in the plasmamembrane. Interrupting this cycle prevents RAS from locating properly. Palmostatin B is a small molecule inhibitor of acyl protein thioesterase (ATP1) that catalyzes depalmitoylation (63). Palmostatin B was able to inhibit *in vitro* growth of murine BM cells with a *Nras* mutation, but had no effect on those with a *Kras* mutation (64). Because the KRAS 4B isoform is not palmitoylated, acyl-protein thioesterase 1 inhibitors would only be applicable to NRAS mutants (3). To our knowledge, there is not any clinical experience with palmostatin B, nor are there studies in animal models.

Targeting downstream signaling pathways

RAF/MEK/ERK pathway

The MEK-inhibitor trametinib has been shown to inhibit aberrant signaling and myeloproliferation *in vitro* using JMML iPSCs, notably with a greater effect on *PTPN11* mutated iPSCs compared to *CBL* mutated iPSCs (65). Trametinib is currently being investigated in a phase 2 clinical trial in patients with relapsed or refractory JMML. Stieglitz and colleagues reported objective responses (1 clinical complete response and 3 clinical partial responses) in 4 out of 9 patients enrolled in this study. Another 2 patients had stable disease troughout the 12 treatment cycles. No molecular responses were achieved. Trametinib also showed a favorable side effect profile (66).

Similarly, the MEK-inhibitor mirdametinib has shown activity in JMML iPSCs with a PTPN11 mutation, while no effect could be appreciated upon exposure of *CBL* mutated iPSCs (65, 67). In *Kras* mutant mice mirdametinib treatment induced a rapid normalization of peripheral blood counts, a rapid improvement in clinical condition and a prolonged survival (68). Unfortunately, two-third of the treated mice succumbed to T-cell acute lymphoblastic leukemia (T-ALL) or T-cell lymphoma after 12 weeks of treatment (68). In contrast, *Nf1* mutant mice were in better clinical condition at the end of the trial compared to the placebo group and no T-ALL development was seen during treatment (69). In phase 1 clinical trials, mirdametinib appeared to be safe and well tolerated (70, 71). Phase 2 clinical trials in neurofibromatosis type 1 (NF1), gliomas and solid tumors are ongoing.

Another strategy targeting the RAF/MEK/ERK-pathway is the use of a DNA enzyme against RAF. A DNA enzyme has the ability to specifically cleave target mRNA and thus inhibit the expression of the corresponding protein. This DNA enzyme against RAF induced substantial inhibition of JMML cell colony formation and an increased survival in a mouse model of JMML (72). However, DNA enzymes are not yet implemented in clinical practice (73).

PI3K/AKT/MTOR pathway

The PI3K/AKT/mTOR pathway is known as a signal transduction cascade and a regulator of a variety of important physiological functions, including cell cycle, cell survival, growth (74).

PI3K is an enzyme with 3 different isoforms: p110α, p110β and p110δ, of which the latter is most abundant expressed in leukocytes and amongst monocytic leukemia samples, thus representing an interesting therapeutic target. Deng et al. illustrated that the PI3K inhibitor pictilisib, demonstrating highest specificity for p110α and p110δ, inhibits growth in *Ptpn11* mutated cells (75). Similarly, *Kras* mutated mice treated with pictilisib clinically improved and had a significantly prolonged survival. Nonetheless, various mice developed T-ALL after treatment (76). In a phase 1 dosing study in patients with solid tumors poor tolerability and limited anti-tumor activity were observed with only occasional objective responses (77). However in other phase 1 studies, pictilisib did show a good safety and tolerability profile and a potential anti-tumor effect (78, 79). To date, there are no clinical trials with pictilisib ongoing.

Another p110δ specific PI3K inhibitor, idelalisib, delayed proliferation of *in vitro Ptpn11* mutated cells and inhibited colony growth in iPSCs with a *PTPN11* or *CBL* mutation. In a *Ptpn11* mutated JMML mouse model, idelalisib significantly prolonged survival (80). Idelalisib is the first FDA-approved PI3K inhibitor for the treatment of relapsed chronic lymphocytic leukemia (CLL) and lymphoma. It has shown good clinical activity as a single agent or in combination therapy. In CLL a series of clinical trials were terminated due to increased risk of death related to infection, however this could not be confirmed in other trials (81-83).

In further attempts to inhibit the PI3K/AKT/mTOR pathway, the AKT-inhibitor MK-2206 was administered to mice with *Kras* mutation and mice with *Nf1* mutation and shown to improve survival, accompanied by a normalization of peripheral blood counts. However, insufficient effect of the compound was observed in clinical studies with various solid tumors and AML and consequentially, further clinical development of the compound has been halted (84).

Finally, mTOR inhibition using rapamycin, a well-known drug for its ability to suppress immunity and in preventing graft versus host disease (GVHD) after organ transplantation and HSCT, was proven to inhibit JMML progression. Two studies have tested this compound *in vitro* in cells derived from patients with JMML. Liu et al. observed inhibition of colony growth in 71% (10/14) of JMML cells *in vitro* treated with rapamycin. In the 4 non-responding samples, a high concentration of PTEN was found (85). Another study, showed similar effects in iPSCs with *PTPN11* and *CBL* mutations (17). In a mouse model with *Ptpn11* mutation, rapamycin ameliorated clinical condition of the mice (86).

A case report documented on a 9-year old patient with JMML experiencing a relapse 9 months after HSCT with development of GVHD which was controlled with rapamycin. Interestingly, 77 months later, the patient was still alive and in remission, raising the possibility that besides the immunomodulating effect, rapamycin also excerted an anti-leukemic effect (87).

Other treatment approaches

Recent studies have discovered novel fusion genes in patients with quintuple negative JMML. In a Japanese cohort, 3 of 16 patients without Ras pathway mutations harboured ALK/ROS1 tyrosine kinase fusions, that were sensitive to ALK inhibition (17). Crizotinib, a small molecule ALK/ROS1 inhibitor, could significantly inhibit colony

growth of *in vitro* cells of two patients with JMML: one with a RANBP2-ALK fusion and one with a DCTN1-ALK fusion. Subsequently, the patient with the RANBP2-ALK fusion was treated with crizotinib and reached complete molecular remission and was succesfully bridged to HSCT. The patient survived without disease recurrence 15 months after transplant (17). Crizotinib also inhibited colony growth of *in vitro* JMML cells with one of the canonical mutations (*CBL*, *PTPN11*, *KRAS*). Furthermore, ALK-inhibitors alectinib, ceritinib and TAE684 inhibited colony growth to the same extent than crizotinib in *in vitro* JMML cells with *PTPN11* mutation.

In addition, Chao and colleagues identified a CCDC88C-FLT3 fusion in a patient with JMML refractory to conventional cytotoxic chemotherapy but sensitive to FLT3 inhibition with sorafenib in monotherapy. After two weeks of treatment with sorafenib, cytogenetic remission was achieved and after 10 weeks, the patient was able to undergo HSCT and was still disease-free after 300 days (88).

Dasatinib, a small molecule tyrosin kinase inhibitor, inhibited colony growth in *in vitro* JMML cells with *PTPN11*, *NF1*, *NRAS* or *CBL* mutations (89). After confirming an *in vitro* sensitivity for dasatinib treatment, a patient with refractory JMML with PTPN11 mutation was treated with dasatinib and reached hematological remission. The patient could undergo HSCT, but eventually died one year later because of relapsed JMML (90). Dasatinib is FDA approved for the treatment of Philadelphia chromosome positive CML and ALL.

Combination therapy

Combinations of targeted therapy could possibly improve treatment effectiveness, inhibition of the disease mechanism on several fronts and dose reduction of the individual drugs. Several cominations of a MEK-inhibitor with other compounds (PI3K-inhibitor, AKT-inhibitor, mTOR-inhibitor, JAK-inhibitor) have been tested in *in vitro* and in mouse models. Generally, the combination therapy had a stronger effect than treatment with a single compound (38, 84, 91-94), although these also might increase the risks of toxicity and side effects.

Current treatment landscape

In this comprehensive overview, it becomes clear that, although a lot of effort has been made to investigate compounds in experimental settings, there is still a huge leap to take for targeted therapy to become a considerable part of the standard of care in JMML. Only azacitidine has recently been approved for newly diagnosed JMML, making this hypomethylating drug a valuable treatment option as bridge HSCT.

Of the targeted therapies in JMML, the MEK inhibitor trametinib shows the most promising results and is under investigation in a phase 2 clinical trial in relapsed and refractory JMML patients. Another MEK inhibitor mirdametinib has recently been approved in a phase 1 clinical trial and potentially has similar effects in JMML.

Besides MEK inhibitors also the JAK inhibitors show potential *in vitro* in JMML, with ruxolitinib being able to stabilize disease in two out of three JMML patients in a phase 1 clinical trial.

Furthermore, the PI3K inhibitor idelalisib is FDA approved for treatment of relapsed CLL and showed activity in iPSCs and a mouse model of JMML.

Rapamycin is well-known for its immunosuppressive effects and is frequently used to limit GVHD after HSCT. In other hematologic malignancies, rapamycin is often used in combination with another compound to sensitize the antileukemic effects. Therefore, combinations with rapamycin could be considered for further research.

In *in vitro* settings, dasatinib and crizotinib were able to inhibit colony growth of JMML cells with a canonical mutation. Both compounds are investigated in various clinical trials and dasatinib is approved for the treatment of Philadelphia chromosome positive CML and ALL. These experiences in other hematologic malignancies could possibly lead to further investigation of these compounds in JMML.

Unfortunately, of these drugs only trametinib is currently under investigation in JMML. As this disease is aggressive with dismal outcomes, other therapies are needed. Importantly, targeted therapies of high interest should always be carefully evaluated within clinical trials. As JMML is rare, international collaboration for this is warranted.

Discussion and conclusion

As JMML is caused by hyperactivation of the Ras pathway, different strategies have been employed to target this pathway, although with varying degrees of success. As the Ras pathway is involved in many biological processes, targeted therapies frequently result in unwanted side-effects, such as the development of T-ALL. Better understanding of the pathophysiology of JMML will help to identify novel therapeutic targets or combinations of existing therapeutic strategies, as demonstrated for long non-coding RNAs and circRNAs (12, 13). Besides HSCT, also other immunotherapeutic approaches may have the potential to cure this disease, as demonstrated by the successful use of donor lymphocyte infusions for post-transplant relapse(95). In 2016, a chimeric antigen receptor (CAR) T-cell targeting the GM-CSF receptor (or CD116) has demonstrated anti-proliferative effects on stem and progenitor cells in JMML (96). Furthermore, although methylation has been studied as a therapeutic target, the role of histone modifications, yet another epigenetic change, remains unexplored in JMML.

In addition, a better understanding of the pathophysiology will not only help to identify and develop novel therapeutic modalities, but also to better pinpoint which specific cell to target in JMML. Recently, integrated analysis combining exome and RNA-sequencing data has discovered JMML-propagating cells and revealed that canonical mutations are acquired in this HSC compartment (27). Surprisingly, besides HSCs also more committed cells such as multipotent progenitors, lymphoid-primed multipotent progenitors, and even common myeloid progenitors and granulocyte-macrophage progenitors are able to propagate the disease in xenograft models (27). Single cell RNA sequencing has shown that all somatic mutations in JMML can be backtracked to the phenotypic haematopoietic stem/progenitor cells compartment with RAS-activating mutations as a “first hit” (97). Moreover, these leukemic stem cells are present after HSCT and before molecular/clinical evidence of relapse (97). This finding paves the way for selective targeting of JMML-propagating cells. In addition to the generation of all afore mentioned data, the collaborative sharing of large-scale datatypes will be essential in further advancing our understanding of JMML disease and moving forward the discovery of novel treatment options. In that respect, the

initiative of Prof. Elliot Stieglitz, providing a central datadeposit and analysis infrastructure for JMML sequencing data (https://www.ncbi.nlm.nih.gov/projects/gapprev/gap/cgi-bin/study.cgi?study_id=phs002504.v1.p1), is of utmost importance.

Besides targeting cell-autonomous features, such as cell surface receptors or intracellular proteins within JMML cells, also non-autonomous mechanisms can be of interest. For instance, it was recently demonstrated that germline *PTPN11* mutations in the BM micro-environment have pathogenic effects on HSCs (98). Patients with somatic *PTPN11* mutations probably have a similarly abnormal BM microenvironment since parts of this niche are haematopoietic driven (99). The pathogenic effect of the mutated BM niche can potentially explain the poor engraftment and high relapse rates after HSCT in JMML. The latter may be due to the inability of the donor HSCs to engraft and remain in a quiescent state in the aberrant BM, with outgrow of the residual leukemic cells as a consequence (100).

For decades, studies in JMML were hampered by the lack of appropriate JMML models. *In vitro* modelling of JMML is very difficult as JMML progenitor cells cannot be maintained in culture and the leukemic clone is lost within a few weeks (101). Therefore, an immortalized JMML cell line, which accurately reflects the lineage diversity of JMML, has not been generated so far and primary patient cells in suspension culture tend to differentiate rapidly. Recently, both patient derived iPSC (65, 67, 102) as well as xenotransplantation (103) have been employed as an alternative approach to model the disease. Hopefully, These novel JMML disease models, will forward development and in vitro and in vivo testing of different new therapeutic options. In conclusion, targeted therapy in JMML is still in its early stage, although different compounds have been tested, of which several show promises. As the patient population amenable for each targeted strategy is rare, it will be crucial to envision international collaborations in clinical trial design. Recent development of JMML models will guide identification of novel therapeutic targets and novel treatment strategies such as targeting JMML-propagating cells, targeting non-autonomous mechanisms and targeting the immunesystem.

Conflict of interest statement

The authors declare no competing interests.

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Legends

FIGURE 1A: a structured overview of targeted therapy in JMML situated on the Ras pathway.

FIGURE 1B: a timeline of compounds studied in JMML. The upper side shows the compounds that have been studied in clinical trials, the underside shows the compounds that have been studied in vitro, in animal models or in case reports. Compounds in gray are considered as abandoned for various reasons: poor safety and tolerability, lack of effect in JMML or other hematologic malignancies, agents that have been taken off market…

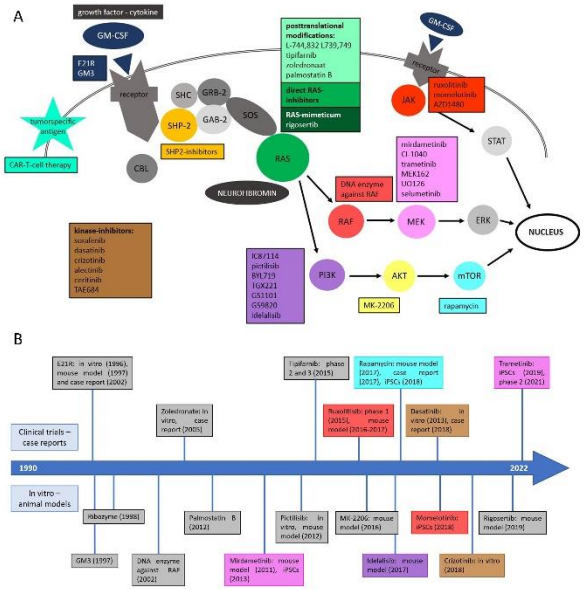

TABLE 1: Overview of all relevant targeted therapies studied in JMML (in vitro, in vivo animal model or in patients with JMML).

Table

TABLE 1: Overview of all relevant targeted therapies studied in JMML (in vitro, in vivo animal model or in patients with JMML).

Drug target	Drug name	Studies	Results	References
GM-CSF signaling pathways				
GM-CSF analog	E21R	*In vitro*	*In vitro*: inhibition of colony growth.	(32-34)
		Mouse model	Mouse model: improved clinical condition.	
		Case report - 1 patient	Case report: temporary response, then relapse.	
DNA triple helix formation	GM3	*In vitro*	*In vitro*: inhibition of colony growth.	(30)
Ribozyme	Ribozyme	*In vitro*	*In vitro: inhibition of colony growth.*	(36)
JAK inhibitor	Ruxolitinib	*In vitro* (iPSCs)	*In vitro*: inhibition of colony growth. More effective with *CBL* mutation than with *PTPN11* mutation	(17, 38-40)
		Mouse model	Mouse model: clinical improvement.	
		Phase 1 clinical study (3 patients)	Phase 1: 2 out of 3 patients temporary stabilization.	
	Momelotinib	*In vitro* (iPSCs)	*In vitro*: inhibition of colony growth. More effective with *CBL* mutation than with *PTPN11* mutation.	(17)
Therapies targeting RAS-MAPK pathway				
RAS mimeticum	Rigosertib	Mouse model	Mouse model: clinical improvement. Prolonged survival.	(52)
Farnesyltransferase inhibitor	L-744,832	*In vitro*	*In vitro*: inhibition of colony growth.	(57, 58)
	L-739,749	Mouse model	Mouse model: no effect.	
	Tipifarnib	Phase 1 clinical study	Phase 1: safe at a dose of 300 mg/m^2.	(59, 60)
		Phase 2/3 clinical study (47 patients)	Phase 2/3: temporary clinical improvement. Survival worse than in the untreated group.	
Bisphosphonates	Zoledronate	*In vitro*	*In vitro*: inhibition of colony growth.	(61, 62)
		Case report (1 patient)	Case report: no effect.	
Palmitoylation - depalmitoylation	Palmostatin B	*In vitro* (mouse)	*In vitro* (mouse): only effective with *Nras* mutation, not with *Kras*.	(64)
Targeting downstream signaling pathways				
MEK inhibitor	Mirdametinib (PD0325901)	*In vitro* (iPSCs)	*In vitro*: inhibition of colony growth. More effective with *PTPN11* mutation than with *CBL* mutation.	(65, 67-69)
		Mouse model	Mouse model: clinical improvement.	
	CI-1040	Mouse model	Mouse model: no effect.	(104)
	Trametinib	*In vitro* (iPSCs)	*In vitro*: inhibition of colony growth. More with *PTPN11* mutation than with *CBL* mutation.	(65, 66)
		Phase 2 clinical study – recruiting	Phase 2: abstract published in november 2021. 4 out of 9 patients had an objective clinical response, from which 1 with a complete response and 3 with a partial response. No molecular responses were achieved(NCT 03190915 (clinicaltrials.gov)).	
DNA enzyme against RAF	DNA enzyme against RAF	*In vitro*	*In vitro*: inhibition of colony growth.	(72)
		Mouse model	Mouse model: clinical improvement, prolonged survival.	

PI3K inhibitor	IC87114 (p110δ)	*In vitro* (mouse)	*In vitro*: inhibition of colony growth.	(75)
	Pictilisib (p110α and p110δ)	*In vitro* (mouse)	*In vitro*: inhibition of colony growth.	(75, 84)
		Mouse model	Mouse model: clinical improvement. Prolonged survival.	
	BYL719 (p110α) + TGX221 (p110β) or GS1101 (p110δ)	*In vitro*	*In vitro*: inhibition of colony growth. Combination with TGX221 or GS1101 twice as effective as BYL719 alone.	(91)
	GS-9820 (p110δ)	*In vitro* (mouse)	*In vitro*: inhibition of colony growth.	(92)
	Idelalisib (p110δ)	*In vitro* (iPSCs)	*In vitro*: inhibition of colony growth.	(80, 92)
		Mouse model	Mouse model: clinical improvement. Prolonged survival.	
AKT inhibitor	MK-2206	Mouse model	Mouse model: clinical improvement. Prolonged survival.	(84)
mTOR inhibitor	Rapamycin	*In vitro* (iPSCs)	*In vitro*: inhibition of colony growth. Linked with concentration of PTEN.	(17, 85-87)
		Mouse model	Mouse model: clinical improvement.	
		Case report (1 patient)	Case report: lasting remission after 77 months.	
Small molecules				
Multikinase inhibitor	Sorafenib	*In vitro* (mouse)	*In vitro*: inhibition of colony growth in cells with a CCDC88C-FLT3 fusion.	(88)
		Case report (1 patient)	Case report: patient with a CCDC88C-FLT3 fusion in cytogenetic remission. 300 days post HSCT fusion transcripts undetectable.	
	Dasatinib	*In vitro*	*In vitro*: inhibition of colony growth (mutation *PTPN11-NF1-NRAS-CBL*).	(89, 90, 105)
		Case report (1 patient)	Case report: patient with *PTPN11* mutation. Hematological remission, bridging to 3th HSCT, died 1 year later from relapse.	
ALK/ROS1 inhibitor	Crizotinib	*In vitro*	*In vitro*: inhibition of colony growth with a DCTN1-ALK fusion, RANBP2-ALK-fusion and *PTPN11-CBL-KRAS* mutations.	(17, 106, 107)
		Case report (1 patient)	Case report: patient with RANBP2-ALK fusion in complete molecular remission. 15 months after HSCT relapse-free.	
ALK inhibitor	Alectinib	*In vitro*	*In vitro*: inhibition of colony growth (cells with *PTPN11* mutation).	(17)
	Ceritinib			
	TAE684			