INTRODUCTION

The mitogen-activated protein kinase (MAPK) pathway is a signal transduction pathway involved in a variety of cellular processes, including regulation of proliferation, survival and differentiation [1], playing a fundamental role in the oncogenesis of various cancers [2] and providing great opportunities to develop novel therapies.

Three major genetic alterations activating the MAPK pathway have been identified: NF1 mutation, BRAF rearrangement and BRAF mutations [3]. Neurofibromatosis type 1 (NF1) is a genetic mutation of NF1 gene resulting in RAS activation. These Individuals are at risk of developing benign and malignant tumors, most commonly cutaneous neurofibromas, plexiform neurofibromas, and optic pathway low-grade gliomas (LGG) [4]

The rearrangement of BRAF and KIAA1549 results in the loss of the BRAF autoregulatory N-terminal domain, causing constitutive activation of BRAF [3]. To date, over 15 different BRAF rearrangements have been identified. BRAF:KIAA rearranged tumors are typically found in pilocytic astrocytomas, most commonly observed in the posterior fossa area [5].

BRAF V600E mutation is the most frequent genetic alteration in the MAPK pathway. In the pediatric population it is most commonly identified in low grade gliomas, high-grade glioma (HGG) ([7]), melanoma [8], Langerhans cell histiocytosis (LCH) , non-LCH, [9] and ameloblastoma [10]

BRAF V600E mutation is considered an actionable target with selective BRAF inhibitors such as Vemurafenib (Zelboraf) and Dabrafenib. Use of BRAF inhibitors is only indicated in BRAF mutated tumors as they may cause paradoxical accelerated tumor growth in BRAF:KIAA rearranged or NF1 mutated tumors[11].

Until recently toxicity reports of these therapies were limited to adults. Dabrafenib has been associated with hyperkeratosis (39%), headache (35%), arthralgia (35%) and pyrexia (32%). In addition, it has been reported that 10% of adult patients develop squamous cell carcinoma. Overall 53% of patients experience side effects at Grade 2 or above, with 28% requiring dose reduction. Still, only 3% of patients have discontinued treatment entirely [12].

The first pediatric phase I/IIa study of Dabrafenib was conducted by Kieran et al. [13]. In this study 26 of 27 patients with BRAF V600E mutant solid tumors (96%) experienced an adverse event (AE) of which 22% experienced grade 3 or 4 AE. The observed toxicity profile was similar to that described in adult patients and included skin toxicities, pyrexia, fatigue, headache, arthralgia, and gastrointestinal events. The most common grade 3 or 4 AEs were arthralgia and maculopapular rash. No patients discontinued treatment for study-drug related AEs, and there were no reports of secondary cutaneous squamous cell carcinoma or drug-related mortality.

Another phase one trial [14] of 32 low-grade glioma patients treated with Dabrafenib showed a similar toxicity frequency (91% all-grade AE; 28% grade3/4 AE). Alongside maculopapular rash and arthralgia, hematological toxicities (DIC and cytopenia) were also observed. No secondary skin malignancies were noted also in this cohort. Treatment was discontinued in two patients as a result of allergy, and arthralgia with erythema nodosum.

MEK inhibitors act further down the molecular pathway and thus are effective in all subtypes of MAPK driven tumors. Trametinib, a highly selective inhibitor of MEK1/MEK2, was the first of this class approved by the FDA in 2013. In adults, rash, diarrhea, fatigue, peripheral

edema and acneiform dermatitis have been reported with Trametinib treatment. The most severe AE of MEK inhibitors are decreased cardiac function (7%), interstitial lung disease (2%) [15] and ocular toxicities (9%) including retinal vein occlusion (RVO) and MEK-associated retinopathy ([15])

Geoerger et al. [16] presented results of their phase I trial in 40 pediatric patients with solid tumors or NF1 plexiform neurofibroma. Among patients treated with Trametinib monotherapy, hyponatremia (n = 2) and pyrexia (n = 2) were the only treatment-related serious adverse events (SAEs) reported in more than 1 patient. A phase II study [17]children treated with Selumetinib, another MEK inhibitor, for NF1 related plexiform neurofibroma observed AEs of rash, GI symptoms and creatine phosphokinase (CPK) elevation at grade 3 or 4. Additionally, one patient suffered from a grade-2 decrease in ventricular ejection fraction not resulting in dose interruption. Overall, a total of fourteen patients had dose reduction for EA (28%) five of these patients (10%) discontinued treatment due to AEs. The aim of the current study is to share a single center experience with MAPK pathway .targeted therapy, focusing on toxicity and management of rare adverse events

METHODS

The institutional Review Board at Sheba Medical Center approved this study.

Twenty-two pediatric patients with molecularly confirmed MAPK pathway driven tumors treated with BRAF and\or MEK inhibitors at the Sheba Pediatric Oncology Department between August 2014 and March 2020 were included in this study. All parents\patients signed a written informed consent and received treatment from Novartis as part of their "compassionate use" program and according to their dosing guidelines.

During treatment patients were evaluated at the following regular intervals, unless more intense follow up was clinically indicated. For the first six weeks, weekly full blood count, blood chemistry, urine analysis, blood pressure and physical exam were performed. Cardiac evaluation (ECG and Echocardiograph) and Ophthalmologic evaluation (ocular exam) were performed Monthly for the first 3 months and then every 2 months. Dermatological evaluation was performed every 4 months. Endocrine evaluation including growth is detailed below.

Drug related toxicity was recorded according to the National Cancer Institute, Common Terminology Criteria for Adverse Events 4.0.

Endocrine evaluation

Endocrine evaluation was performed prior to initiating treatment and thereafter every 3-6 months as indicated. Height, weight, and BMI standard deviation scores were calculated using age and sex-specific growth data (based on the Centers for Disease Control and Prevention's Year 2000 Growth Charts) found adequate for assessing Israeli children and adolescents ([18]). Pubertal development was assessed using the Tanner scale and laboratory tests including Gonadotropins and sex hormones. Thyroid function tests and Cortisol were also measured.

Data Analysis

The initial analysis included estimations of mean, standard deviation and frequency distribution. Comparison between values at base line and at the end point was made using paired t test. Results were considered statistically significant if the two-sided p value was below 0.05. Calculations were performed using SPSS statistics 25.0 (IBM Armonk, NY, USA) a statistical software package. The univariate odds ratio for risk of major adverse

events and dermatological adverse events was compared between patients treated with Dabrafenib compared to those treated with Trametinib and calculated using a logistic regression model. For this analysis, patients treated with a combination of both drugs (n=4) were excluded.

RESULTS

Twenty-two patients, of whom seven were female (32%), with a variety of histological diagnoses were included in this study, Treatment included BRAF inhibitors, mostly Dabrafenib (n=11), the MEK inhibitor Trametinib (n=7) or both (n=4). The average age at treatment initiation was 10.7 years (range 3-19 years). Nine patients received MAPK inhibitors as upfront therapy (surgery excluded). Mean treatment duration was 23 months (range 3-46 months). Patient characteristics are depicted in *TABLE 1*.

TABLE 1 – PATIENT DEMOGRAPHIC CHARACTERISTICS

Adverse Events

Overall an adverse events rate of 86% was encountered. AEs are detailed in table 2 and table 3. Dermatological disorders accounted for 68% of all adverse events. Eight patients (35%) suffered a severe adverse event; osteoporosis with a pathological fracture, Sarcoid-like massive lymphadenopathy, retinal pigment epithelial changes, grade 4 elevated liver enzymes , grade 3 CPK elevation, grade 3 rash, and erythema nodosum in two patients. The prevalence of severe adverse events was similar in the Dabrafenib and Trametinib groups. Treatment was discontinued temporary in clinically severe or CTCAE Grade 3 & 4 adverse events and

renewed according to Novartis guidelines. Four patients permanently discontinued treatment as a result of toxicity, of which one patient on combination therapy continued Dabrafenib as a single agent following retinal pigment epithelial changes.

TABLE 2 – ADVERSE EVENTS BY DRUG

Dermatological, Hair & Nail Sequelae

Dermatological sequalae were the most common adverse events. Fifteen patients (68%) presented with cutaneous lesions, hair or nail changes.

In this pediatric cohort the most common manifestation was acneiform rash, which occurred in seven patients (31%). One patient had a CTCAE grade 3 pityriasis lichenoides et varioliformis acuta (PLEVA) like rash that required temporary cessation of treatment. No patients developed SCC.

Paronychia was reported in four patients, all of whom were receiving Trametinib. In total 60% of patients treated with Trametinib suffered from recurrent paronychia in multiple nails whereas no patients treated with Dabrafenib or on combination therapy reported this adverse effect.

Three patients treated with Dabrafenib reported hair transformation from straight to curly.

.One additional patient treated with Trametinib suffered from new-onset significant hair loss

Systemic Inflammatory events

Pyrexia

Two patients had treatment related febrile episodes without evidence of infection cause. One patient treated with Dabrafinib. The other patient treated with Trametinib, had a febrile episode accompanied by elevated LDH.

Erythema Nodosum

Two patients, treated with Dabrafenib and Zelboraf respectively, suffered from erythema nodosum that resolved following cessation of treatment. One patient had a repeat reaction upon re-challenge and the treatment was then stopped definitively.

Sarcoid-like Massive Pulmonary Lymphadenopathy

One patient, a 7-year-old boy diagnosed with a BRAF V600E mutated brainstem ganglioglioma was treated with Dabrafenib at a dose of 125mg twice daily. Three weeks after commencement of treatment he presented with stridor, fever, dyspnea and oxygen desaturation. On chest x-ray a new diffuse bilateral pneumonia was noted. Treatment with Azithromycin and Co-Amoxiclav was started and Dabrafenib treatment was stopped. In light of continued clinical symptoms chest CT was performed. Diffuse bi-hilar lymphadenopathy was observed, compressing the main bronchi and causing secondary atelectasis in addition to bilateral pulmonary infiltrates (see *FIGURE 1*). Due to progressive respiratory difficulty steroid treatment was initiated. Infectious disease work-up was negative including blood cultures, respiratory viral work-up and lymph node biopsy, which was negative for bacteria, fungi & mycobacteria on stain and culture. The biopsy was performed after 4 days of steroid therapy. The pathology was highly suggestive of a reactive lymph node, and sarcoidosis or other granulomatous disease was excluded. Following clinical and radiological improvement Dabrafenib treatment was renewed at a lower dose in combination with steroid therapy. An attempt at steroid weaning resulted in a second episode of respiratory distress accompanied by pneumonitis and signs of hilar lymphadenopathy on CT. Consequently, treatment with Dabrafenib was stopped.

FIGURE 1: MASSIVE POLMUNARY LYMPHADENOPATY

Endocrine Evaluation

Four patients were excluded from this analysis due to insufficient information (treatment started at another center or short duration of follow up).

Linear Growth

Of the 18 patients evaluated, three patients had already completed their linear growth prior to treatment with MAPK pathway inhibitors. Eight patients had a normal growth rate during treatment. One patient, diagnosed with growth hormone excess, had an accelerated growth rate both before and during treatment.

No statistically significant growth impairment was noted, but Six patients had growth impairment during treatment. Two patients had significant growth retardation during prolonged (31 and 36 months) Dabrafenib treatment (Figure 2). One of them (N# 5) had a decrease in height-SDS from 0.16 before treatment to -1.0. without any other known risk factors for growth retardation, while in the other patient, with an hypothalamic involvement of JPA that might partially explain growth retardation (number 3) had a decrease in height-SDS from 1.35 to -0.11, both patients had normal pituitary function.

The other 4 patients with slow growth rate have considerable comorbidities that influence growth significantly. Two patients had radiation-induced growth hormone (GH) deficiency and were previously treated with GH, which was discontinued due to tumor recurrence prior to treatment with MAPK pathway inhibitors. During treatment with Trametinib growth rate in both patients was particularly slow (1cm/year). Notably, one patient was treated with a GnRH analogue and the other had delayed puberty, which could also have contributed to growth deceleration. Two additional patients had a mild deceleration in growth rate but further follow-up is needed in order to establish this finding.

Sexual Maturation

In this small cohort we did not encounter any treatment-related abnormalities of sexual maturation or gonadal function.

Of the 18 patients evaluated, four patients were prepubertal throughout treatment duration, which was appropriate for their age. Seven patients progressed through puberty as expected, with a normal hormone profile. Of these, four were prepubertal at treatment initiation and entered puberty age appropriately during treatment. One patient had been treated with a GnRH analogue for precocious puberty prior to Trametinib initiation. Two patients completed their pubertal maturation prior to treatment and did not have any hormonal abnormalities indicating gonadal insufficiency during treatment.

One patient with a primary hypothalamic LGG showed signs of precocious puberty whilst being treated with Dabrafenib. This was assumed to be related to her hypothalamic tumor and not to her treatment.

Two patients had delayed sexual maturation during treatment. One patient had growth hormone deficiency secondary to prior brain irradiation and LGG with hypothalamic involvement. The other patient had a family history of delayed sexual maturation.

Bone Health

A 16-year-old boy diagnosed with a BRAF/PIB translocated low grade sarcoma of the cervical spine, started treatment with Trametinib due to local progression in the context of an inoperable tumor. Six months following commencement of Trametinib therapy he underwent PET-CT demonstrating a right sided sacral stress fracture. A dual-energy X-ray absorptiometry (DEXA) scan reported Z-scores consistent with low bone mineral density for his age (lumbar spine, –3.2, total body -1.1) with a trabecular bone score (TBS) of 1.327. He

was treatment with Zolendronic acid and Trametinib treatment was discontinued. The patient had no risk factors for osteoporosis such as prior treatment with glucocorticoids or family history. Endocrine workup excluded known causes of secondary osteoporosis: serum calcium, phosphorus, alkaline phosphatese, and magnesium, as well as PTH, thyroid function tests, LH, FSH and testosterone levels were all normal. 25-hydroxy vitamin D level was 19.9ng/ml. Serology for celiac disease was negative and he had no clinical features suggesting inflammatory bowel disease as primery reasons for osteoporosis.

Ocular Toxicity

One patient experienced severe ocular side effects – an 11-year-old girl diagnosed with PXA grade 3 harboring BRAFV600E and CDK2NA Deletion. She was started on combination therapy with Dabrafenib at 150mg daily and Trametinib at 0.7mg daily. Ocular exam including baseline optical coherence tomography (OCT) was normal. One month into treatment bilateral macular retinal lesions were noted (*FIGURE 3*). These lesions were not present in the baseline OCT scans. The patient was asymptomatic but due to bilateral macular involvement, as opposed to typical MEK inhibitor retinopathy (MAKER) morphology, this retinopathy was judged to be a severe adverse event and treatment with Trametinib was stopped. Dabrafenib was renewed as a monotherapy. Lesions remained unchanged and the patient remained asymptomatic with 20/20 vision in both eyes. Visual fields (including 10-2 fields) did not demonstrate macular scotomas with the limit of reduced reliability.

FIGURE 3

Left ventricular function

All patients had age and weight appropriate left ventricular function, measured as M-mode left ventricular fractional shortening (LVFS) [19] before and during treatment. However, on retrospective review of patient's Echocardiography results, non-symptomatic variations in SF

% during treatment were noted in 2 patients. These were two adolescent patients treated with Trametinib. One patient (#19), demonstrated mild reduction in LVFS during the first 3 months of treatment (39% to 28%). Shortly after treatment cessation, due to grade 3 CPK elevation (with normal troponin), LVFS improved to 34%. After restarting the treatment at full dose SF decreased to 30%. Upon cessation of treatment the LVFS returned to baseline level of 37%. A similar trend was noted in a second patient - Baseline LVFS- 39%, 28% on full dose with an increase to 36% on half dose

TABLE 3 – ADVERSE EVENT FREQUENCY

Other Toxicities

One patient treated with Zelboraf had a grade 4 ALT\AST elevation and grade 3 hyperbilirubinaemia that resolved with cessation of treatment and did not recur after switching medication to Dabrafenib.

Four of the seven patients treated with Trametinib showed CPK alterations, one of which at grade 3 level. None of the patients suffered from disturbance of renal function or clinical signs of rhabdomyolysis. Dose reduction or treatment cessation was initially performed in patients with grade 3-4 CPK elevation.

DISCUSSION

This pediatric cohort included 22 patients with a variety of MAPK driven tumors treated with MAPK inhibitors. Overall an AEs frequency of 86% was encountered. Dermatological disorders accounted for 68% of the adverse events, results similar to those of other pediatric groups [20]. Interestingly, only one of the patients treated with combination therapy suffered a dermatological AE in contrast to all seven patients treated with Trametinib alone. It appears

from adult clinical trails ([21]) that combination BRAF and MEK inhibitor therapy results in reduced cutaneous toxicity when compared with monotherapy.

Eight patients (36%) suffered a CTCAE grade 3 or 4, or clinically severe adverse event and had to discontinue treatment. Of these, five patients required only a temporary cessation of treatment, including one patient on combination therapy who continued Dabrafenib as a single agent, while three patients completely discontinued treatment.

Evaluating linear growth in this cohort of patients is challenging, as many of them have multiple risk factors for growth impairment, including hypothalamic or pituitary tumors, hormone deficiencies, prior brain irradiation and treatment with GnRH analogues.

Two patients in the cohort had significant growth retardation during Dabrafanib treatment despite having no endocrine or treatment-related risk factors for impaired growth. Two additional patients, known to have growth hormone deficiency, had complete growth arrest during Trametinib treatment. Slow growth may be explained by the interconnection of RAS–MAPK signaling with the GH signaling cascade. It is also known that patients with germline mutations of components of the RAS–MAPK signaling pathway, such as KRAS, RAF1, BRAF and patients with Noonan syndrome (PTPN11 mutation) suffer from growth failure [22]. Although no statistically significant growth impairment was noted, till a larger study will shed light on this subject, we recommend close follow up by a pediatric endocrinologist in order to detect changes in growth pattern and sexual maturation during treatment.

One adolescent patient presented with osteoporosis with a pathological bone fracture and a markedly decreased bone mass. He had no other risk factors for osteoporosis, and endocrine evaluation did not identify any other cause for secondary osteoporosis. Case reports suggest a causal relationship between MEK inhibitors and osteoporosis ([23]) Several studies have shown that the ERK-MAPK pathway promotes osteoblast differentiation and bone formation in vitro and in vivo [24]

In this cohort severe retinal toxicity was encountered in one patient soon after commencement of combination therapy. On fundoscopy an acute macular neuroretinopathy (AMN) was diagnosed, this entity, considered a rare form of perifoveal photoreceptor damage and has been previously associated, among other causes, with treatment with atezolizumab – an anti PD-L1 ([15,25] In order to detect smaller lesions we now routinely .perform dense macular OCT at base line and on every ocular evaluation thereafter

Inflammatory adverse events occurred in three patients treated with Dabrafenib; Erythema .Nodosum in two patients and a Sarcoid-like pulmonary lymphadenopathy

Erythema Nodosum (EN) is an acute, nodular, erythematous eruption usually limited to the extensor aspects of the lower legs presumed to be a hypersensitivity reaction occurring in association with systemic disease, drug therapy or idiopathic. Sarcoid-like granulomatous reactions have been reported in patients receiving antineoplastic biological treatment, most commonly immunotherapy as well as treatment with BRAF and MEK inhibitors [23 26,27].
The mechanism by which BRAF inhibitors activate a systemic inflammatory response is being studied. It appears that treatment of melanoma cells *in vitro* with BRAFV600E inhibitors results in elevated presentation of melanoma-associated antigens, irrespective of the melanoma BRAF mutation state [28]. Furthermore, effector T cells have been reported to be stimulated via so-called paradoxical activation of ERK signaling by BRAFV600E inhibitors [29]. In addition, tumor specimens of patients under treatment with MAPK inhibitors showed increased infiltration with cytotoxic T cells [29]. It is possible that BRAF inhibitors have an immunomodulatory effect that is yet to be well defined [30].
No patients experienced clinically significant cardiac side effects. Although left ventricular fractional shortening remained within normal range for age for all patients, dose-dependent SF decrease was noted in two patients.

No hematological toxicity, including neutropenia, and no severe infections including COVID-19 were encountered (August 2020). During the COVID-19 outbrake, following Israeli Pediatric Association guidelines, these patients continued attending school and engaging in social activity, as opposed to patients undergoing chemotherapy who were considered immunocompromised.

Overall, we found a toxicity profile similar to that reported in prior studies, however in this cohort we evaluated potential effects on growth pattern and sexual maturation that have not been previously reported and cannot be extrapolated from adult data.

We acknowledge that this cohort includes a mix of histological diagnoses, and combined information on Dabrafenib and Trametinib.

CONCLUSIONS

Our cohort exemplifies some of the uncommon but complex to manage severe adverse events that may be encountered during treatment with MAPK inhibitors in pediatric patients. Only 4 patients stopped treatment as a result of toxicity.

The pediatric oncology community struggles to reduce the side effects of chemo- and radiotherapy and is in search of alternative treatments. Conversely the unknown long-term toxicity of experimental drugs is of great concern. The effect that these treatments might have on growth, cognitive development and sexual maturation, including fertility, remains under-studied.