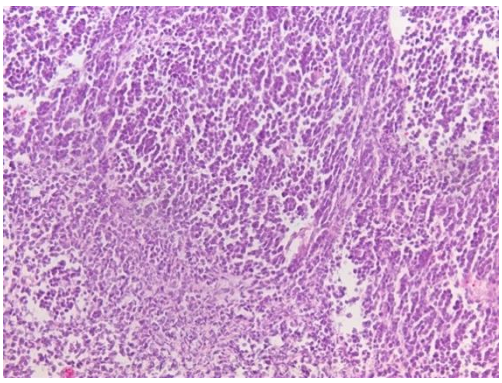
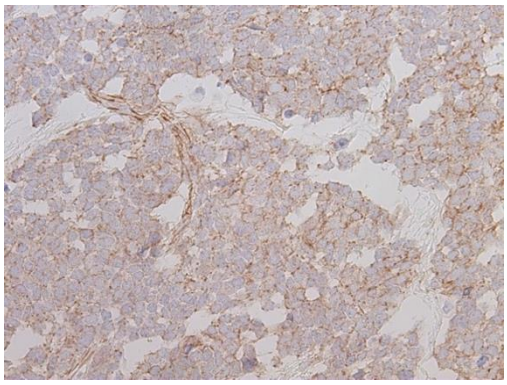
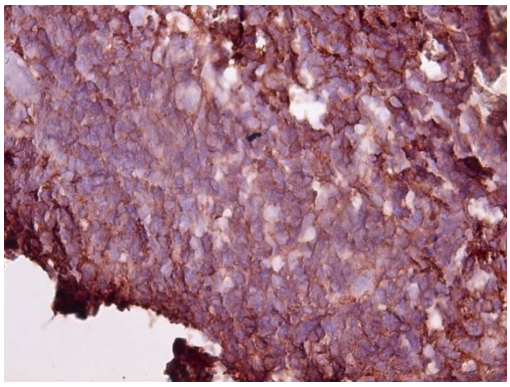
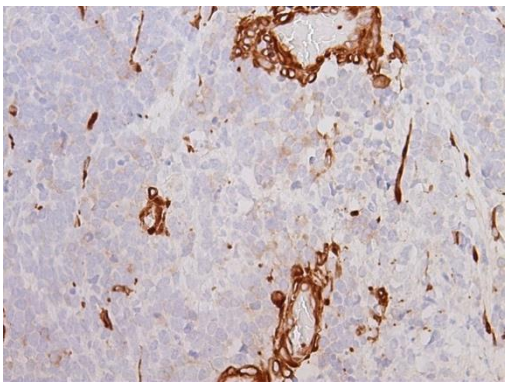
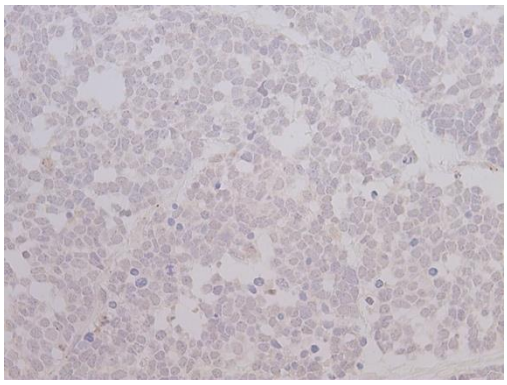
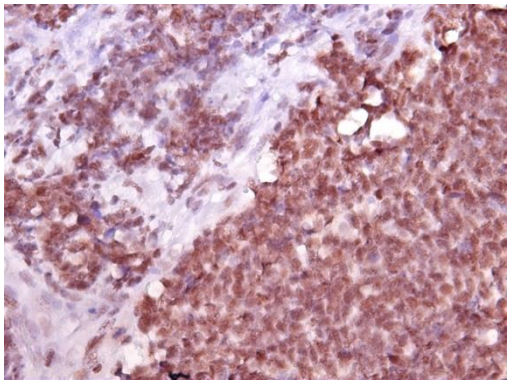
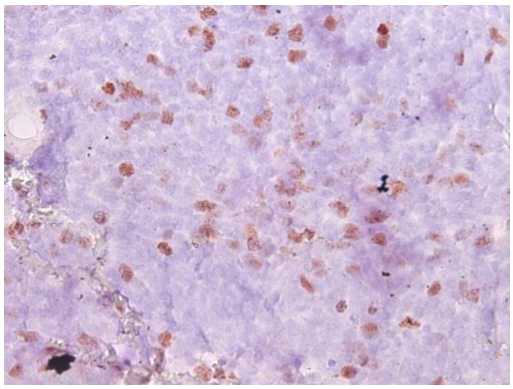
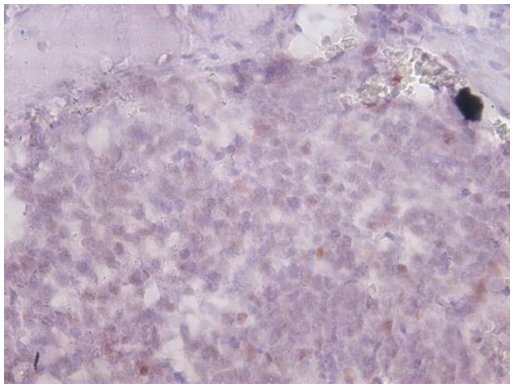
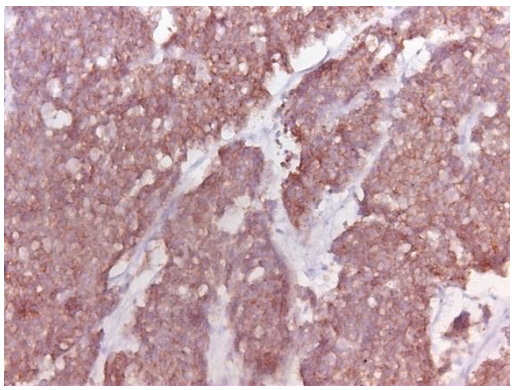
	Hematoxylin/eosin x200
	Beta-catenin x400 Beta catenin monoclonal antibody [(CAT-5H10); cat. no. 13-8400; dilution, 1:50; Rosmedbio Ltd.]
	CD56 x400 Recombinant anti-NCAM1 antibody (EPR2566; cat. no. ab133345; dilution, 1:200; Dia-M Ltd.)
	Filamin A x400 Recombinant anti-filamin A antibody (EP2405Y; cat. no. ab76289; dilution, 1:200; Dia-M Ltd.)

	GAB1 x400 Phospho-GAB1 (Tyr659) polyclonal antibody (cat. no. PA5-36908; dilution, 1:100; Rosmedbio Ltd.)
	INI1 x400 Recombinant anti-SNF5/SMARCB1 antibody (EPR12014-77; cat. no. ab192864; dilution, 1:500; Dia-M Ltd.)
	ki67 x400 Recombinant anti-Ki67 antibody (EPR3610; cat. no. ab92742; dilution, 1:500; Dia-M Ltd.)
	p53 x400 Recombinant anti-p53 antibody (SP5; cat. no. ab16665; dilution, 1:100; Dia-M Ltd.)

	Synaptophysin x200 Recombinant anti-synaptophysin antibody (YE269; cat. no. ab32127; dilution, 1:500; Dia-M Ltd.)

Supplementary File S2. Protocol of metronomic chemotherapy (MCT) with sirolimus in heavily pretreated pediatric patients with embryonal brain tumors: Treatment plan, inclusion/exclusion criteria, response criteria, toxicity and efficacy evaluation and dose modification

I) Inclusion criteria for patients

1. Relapsed or refractory tumors of the central nervous system (all morphological variants according to the WHO classification), in the absence of other options for antitumor therapy.

2. Histological verification of a malignant tumor of the central nervous system [exception: Brain stem tumors, the presence of positive tumor markers -AFP, hCG (blood, CSF)].

3. Low life expectancy.

4. Age: 12 months - 18 years.

5. Karnofsky performance status ≥50% (for children >12 years of age); Lansky play scale ≥50% (for children ≤12 years).

6. Resolution of toxicity after previous antitumor therapy.

7. Participants must have normal organ (liver, kidneys, lungs) and bone marrow function.

8. Written informed consent of patients and/or parents.

II) Exclusion criteria for patients

1. Pregnancy or breast feeding.

2. Use of drugs that are CYP3A4 inducers or inhibitors.

4. Use of other palliative chemotherapy regimens/any other methods of antitumor therapy.

5. Active infection.

6. Lack of adequate laboratory monitoring of the parameters set by the therapy program.

7. Known intolerance to any drugs in the protocol.

III) Diagnostics

1. Clinical data

- Medical history, assessment of physical condition;

- Assessment of previous antitumor therapy (all options used: Surgical treatment, total radiation therapy, radiation fields, chemotherapy-total doses of cytostatics, intraventricular therapy);

- Height/weight, body surface;

- Neurological status;

- Routine physical examination;

- Karnowski status/ Lansky (for children <12 years of age).

2. Laboratory investigations

Weekly (+/- 3 days):

- Full blood count with the leukocyte formula (white blood cells, absolute neutrophil count, lymphocytes, hemoglobin, platelet count);

- Clinical chemistry: ALT/AST, total bilirubin with fractions, creatinine, urea, sodium, potassium;

- Monitoring of CRP level, since patients may not develop fever or pain under continuous treatment with celecoxib (NSAIDs);

- Monitoring of the Sirolimus level in the blood serum (reference values: 10-15 ng/ml).

Monitoring of infection status (high risk of opportunistic infections due to prolonged immunosuppression, lympho- and neutropenia)!

Every two weeks (+/- 3 days):

- Urinalysis;

- Clinical chemistry: Calcium, magnesium, phosphorus, GFR.

Monthly (+/- 1 week):

- Pregnancy test in pubertal females;

- Blood PCR for viruses (CMV, herpes viruses 12, 6 types, parvovirus B19).

Every 3 months (+/- 2 weeks):

- Cytological examination of cerebrospinal fluid;

- Hormones: TSH, T4, T3, cortisol;

- Audiogram

- Ultrasound of the abdominal cavity, retroperitoneal space, small pelvis;

- Echocardiography;

- Neurologist, ophthalmologist; endocrinologist, cardiologist exams - according to indications.

3. Imaging methods of examination

MRI of the brain and spinal cord with and without contrast enhancement

- Within 2 weeks before the start of MCT;

- In the case of repeated surgical treatment, a post-operative MRI obtained within 72 h after surgery;

- Assessment of disease response to therapy - every 12 weeks;

- MRI 6 months after the start of treatment (week 24-28) is mandatory;

- MRI outside the specified time frame is performed according to indications: Deterioration of the neurological status, intolerance to therapy, etc.

MSCT of the abdomen and chest in order to exclude metastatic damage

- Before the start of therapy;

-1 time in 6 months or earlier in case of negative dynamics.

Osteoscintigraphy (only for patients with suspected bone damage) or ^{18}F-FDG.

IV. Treatment response criteria

1. Cytological criteria:

Complete response (CR): absence of tumor cells in the cerebrospinal fluid (2 negative results with a 2-week study interval);

Stable disease (SD): Failure to achieve negative CSF cytology;

Progressive disease (PD): Appearance of tumor cells in the cerebrospinal fluid (double examination) after 2 consecutive negative results.

2. Radiological criteria:

CR: Complete disappearance of tumor signs according to 2 studies at least 4 weeks apart; no tumor cells in the CSF

Partial response (PR): Reduction of tumour volume >50% (in the sum of the volume of all measurable lesion) according to 2 studies at least 4 weeks apart; lack of synchronous progression of any foci or the appearance of new ones. Patients with absence of tumor cells in the cerebrospinal fluid should remain in the same status;

SD: Reduction of tumour volume ≤50% or increase ≤25%; no evidence of new lesions.

PD: Increase of tumour volume >25% or new lesions; appearance of tumor cells in the cerebrospinal fluid.

No recurrence: No tumor foci in patients with total tumor resection, no new ones.

Follow-up should be continued during the entire period of anticancer therapy and for 5 years after its completion with an assessment of imaging data and delayed toxicity.

V. Treatment plan

Drug	**Route of administration**	**Treatment duration**
Sirolimus (Rapamune, Rapamycin)	Initial dose: 2 mg/m^2/day once orally, daily Target concentration in blood serum: 10-15 ng/ml Dose adjustment until target blood levels are reached.	1 cycle = 6 weeks The total duration of therapy is 16 cycles or 2 years Stop therapy if side effects and/or tumor progression occur
Celecoxib	Children <10 kg: 50 mg twice a day orally, daily Children 10-35 kg: 100 mg twice a day orally, daily Children >20 kg: If well tolerated, dose escalation to 200 mg twice a day orally, daily Children >35 kg: 200 mg twice a day orally, if well tolerated, dose escalation to 300 mg twice a day orally, daily Children >50 kg: If well tolerated, dose escalation to 400 mg twice a day orally, daily *Dose escalation may be carried out every 1-2 weeks or according to tolerance.* *Dose de-escalation is carried out up to the maximum well tolerated.*	The total duration of therapy is 16 cycles or 2 years; Stop therapy if side effects and/or tumor progression occur
Etoposide	Dose: 50 mg/m^2/day (max 100 mg) once orally, daily Alternation with cyclophosphamide	1 cycle (6 weeks) = 1st to 2nd weeks of each cycle. The total duration of therapy is 4-5 cycles or upon reaching A total cumulative dose of 2,100 mg/m^2 (4-5 courses of therapy) Stop therapy if side effects and/or tumor progression occur
Cyclophosphamide	Dose: 2,5 mg/kg/day (max 100 mg) once orally, daily Alternation with etoposide	1 cycle (6 weeks) = 4th to 6th weeks of each cycle. The total duration of therapy is 16 cycles or 2 years Stop therapy if side effects and/or tumor progression occur

VI. Dose modification

• *Initiation of cyclophosphamide/etoposide therapy at 100% dose:*

-ANC ≥1,500/mm^3/mm^3

- Thrombocytes/platelets ≥50,000/mm^3

-Total bilirubin <25,5 mmol/l

- Creatinine <1,5xULN

• *Dose reduction of cyclophosphamide/etoposide up to 70%:*

-ANC 750-<1,500/mm^3

-Platelets 30-50,000/mm^3

• *Discontinuation of cyclophosphamide/etoposide until hemogram parameters are restored:*

- ANC <750/mm^3

- Platelets <30,000/mm^3

Missed doses are not made up

VI.1. Etoposide

In case of intolerance to a dose of 50 mg/m^2/day - reduce Etoposide to 35 mg/m^2/day.

In case of haematological toxicity, see point III for dose modification.

Stop therapy upon reaching a total cumulative dose of 2,100 mg/m^2 (4-5 courses of therapy).

VI.2. Cyclophosphamide

In case of intolerance to a dose of 2.5 mg/kg/day - reduce Cyclophosphamide to 0.5 mg/kg/day.

In case of haematological toxicity, see point III for dose modification.

Individual registration card of the patient

Registration form

Full Name

Gender male ☐ female☐

Date of Birth | | | | | | | Date of hospitalization | | | | | | |

Date of diagnosis | | | | | | | Date of start therapy | | | | | | |

INITIAL DATA

Height _____ cm Weight _____ kg

History of disease and treatment

age at onset of disease	
residual tumor	
shunt surgery	
repeated surgeries	
initial stage of the disease	
histological diagnosis	
immunohistochemical profile	
molecular genetics profile (if available)	
date of initiation of 1st line anticancer therapy	
therapy protocol (name)	
total etoposide dose for the entire treatment period	
radiotherapy,doses	
type of radiation therapy	
therapy end date	

disease status after completion of therapy	
anti-relapse therapy	
therapy line	

Status assessment at the time of initiation of therapy

age at relapse	
repeated surgeries	
disease stage	
histological diagnosis	
immunohistochemical profile	
molecular genetics profile (if available)	

Monitoring of disease status during therapy

	start of therapy	**3 months**	**6 months**	**individual evaluation terms**
date				
MRI of the brain with contrast (disease status)				
MRI of the spinal cord with contrast (disease status)				
PET/CT with methionine (optional)				
Cytological examination of the cerebrospinal fluid (tumor cells)	YES □ NO□	YES □ NO□	YES □ NO□	YES □ NO□
Neurological status (improvement/deterioration)				

Discontinuation of therapy in case of progressive disease YES □ NO□

Date of discontinuation of therapy | | | | | | |

Evaluation of therapy toxicity
To be completed only for an episode of severe toxicity (including infections)

Full Name _____

Gender male □ female□

Start of an infectious episode (date) | | | | | | | End of an infectious episode (date) | | | | | | |

Week of ongoing anticancer therapy _____

Cancellation of anticancer therapy YES □ NO□

Toxicity variant _____

Type of infection: bacterial □ viral □ fungal□

Site of infection: _____

Supportive care (list) _____

_____

Continuation of therapy YES □ NO□

Date of resumption of therapy | | | | | | |

Evaluation of therapy toxicity (performed every 6 weeks)

Full Name

Gender male □ female□

Week of ongoing anticancer therapy: 6th □ 12th □ 18th □ 24th□

Table 1. Possible adverse events and criteria for evaluation

	0	1	2	3	4
general state	norm	mild	moderate	severe	life-threatening
hemoglobin	norm	10.0 - < N	8.0 - < 10.0	6.5 - < 8.0	< 6.5
leukocytes	norm	3.0 - < 4.0	2.0 - < 3.0	1.0 - < 2.0	< 1.0
neutrophils	norm	1.5 - < 2.0	1.0 - < 1.5	0.5 - < 1.0	< 0.5
platelets	norm	75 - < 100	50 - < 75	10 - <50	< 10
ALT / AST	norm	> ULN - 2.5*ULN	> 2.5-5* ULN	> 5-20* ULN	> 20* ULN
bilirubin	norm	> ULN - 1.5* ULN	> 1.5-3* ULN	> 3-10* ULN	> 10* ULN
nausea	No	maybe have	oral intake is significantly reduced	hardly eats	parenteral nutrition
vomiting	No	1 episode / day	2-5 episodes per day	> 6 episodes/day	parenteral nutrition
stomatitis	No	painless ulcer/erythema	painful ulcer/erythema, may have	painful ulcer/erythema, cannot eat	parenteral nutrition
diarrhea	No	2-3 episodes / day	4-6 episodes/day, nightl stools, spasms	7-9 episodes/day, incontinence	≥10 episodes or parenteral nutrition
lymphocele	No	asymptomatic course	the presence of symptoms; indicated medical intervention	severe symptoms; invasive intervention is indicated	-
headache	No	slight pain	moderate pain; limitation of daily activities	severe pain, limitation of self-care	-
fever	No	38.0 - 39.0 ° C	> 39.0 - 40.0 ° C	> 40.0 ° C less than 24 hours	> 40.0 ° C > 24 hours
rash	No				
CNS - toxicity	No	temporary lethargy	somnolence <50% of the time, moderate disorientation	somnolence ≥50% of the time, hallucinations	coma, convulsions
Peripheral neurotoxicity	No	paresthesia	deteriotation of previous paresthesia and / or moderate weakness	severe paresthesias leading to motor disturbances	paralysis