Day One Biopharmaceuticals

ASCO 2023 Conference Call

June 2023

Forward-Looking Statements

This presentation and the accompanying oral commentary contain forward-looking statements that are based on our management's beliefs and assumptions and on information currently available to our management. Forward-looking statements are inherently subject to risks and uncertainties, some of which cannot be predicted or quantified. In some cases, you can identify forward-looking statements by terminology such as "may," "will," "should," "could," "expect," "plan," anticipate," "believe," "estimate," "predict," "intend," "potential," "would," "continue," "ongoing" or the negative of these terms or other comparable terminology. Forward-looking statements include all statements other than statements of historical fact contained in this presentation, including information concerning our future financial performance, including the sufficiency of our cash, cash equivalents and short-term investments to fund our operations, business plans and objectives, timing and success of our planned nonclinical and clinical development activities, timing and results of nonclinical studies and clinical trials, efficacy and safety profiles of our product candidates, execution of the Phase 2 clinical trial for tovorafenib and the Phase 1b/2 clinical trial for tovorafenib and pimasertib as designed, any expectations about safety, efficacy, timing and ability to complete clinical trials and to obtain regulatory approvals for tovorafenib and other candidates in development, the ability of tovorafenib to treat pediatric low-grade glioma (pLGG) or related indications, the potential therapeutic benefits and economic value of our product candidates, potential growth opportunities, competitive position, industry environment and potential market opportunities, our ability to protect intellectual property and the impact of global business or macroeconomic conditions, including as a result of inflation, rising interest rates, instability in the global banking system, and geopolitical conflicts, including the war in Ukraine, on our business and operations.

Forward-looking statements are subject to known and unknown risks, uncertainties, assumptions and other factors. It is not possible for our management to predict all risks, nor can we assess the impact of all factors on our business or the extent to which any factor, or combination of factors, may cause actual results to differ materially from those contained in any forward-looking statements we may make. These factors, together with those that are described under the heading "Risk Factors" contained in our most recent Quarterly Report on Form 10-Q filed with the Securities and Exchange Commission ("SEC") and other documents we file from time to time with the SEC, may cause our actual results, performance or achievements to differ materially and adversely from those anticipated or implied by our forward-looking statements.

In addition, statements that "we believe" and similar statements reflect our beliefs and opinions on the relevant subject. These statements are based upon information available to us as of the date of this presentation, and although we believe such information forms a reasonable basis for such statements, such information may be limited or incomplete, and our statements should not be read to indicate that we have conducted a thorough inquiry into, or review of, all potentially available relevant information. These statements are inherently uncertain and investors are cautioned not to unduly rely upon these statements. Furthermore, if our forward-looking statements prove to be inaccurate, the inaccuracy may be material. In light of the significant uncertainties in these forward-looking statements, you should not regard these statements as a representation or warranty by us or any other person that we will achieve our objectives and plans in any specified time frame, or at all. We undertake no obligation to publicly update any forward-looking statements, whether as a result of new information, future events or otherwise, except as required by law.

This presentation also contains estimates and other statistical data made by independent parties and by us relating to market size and growth and other data about our industry. This data involves a number of assumptions and limitations, and you are cautioned not to give undue weight to such estimates. In addition, projections, assumptions and estimates of our future performance and the future performance of the markets in which we operate are necessarily subject to a high degree of uncertainty and risk.

Day One Participants

Jeremy Bender, PhD, MBA
Chief Executive Officer

Samuel Blackman, MD, PhD
Co-Founder and Head of R&D

Charles York, MBA
Chief Operating & Financial Officer

Cancer Drug Development For People Of All Ages

Mission That Creates Value

- Day One's mission is to help children with cancer, from day one and every day after
- Develop medicines for genomically-defined cancers
- Establish first-in-class position through rapid pediatric registration
- Expand to adolescent and adult populations in parallel and pursue those opportunities with the same commitment we do for children

Tovorafenib (DAY101) Lead Program

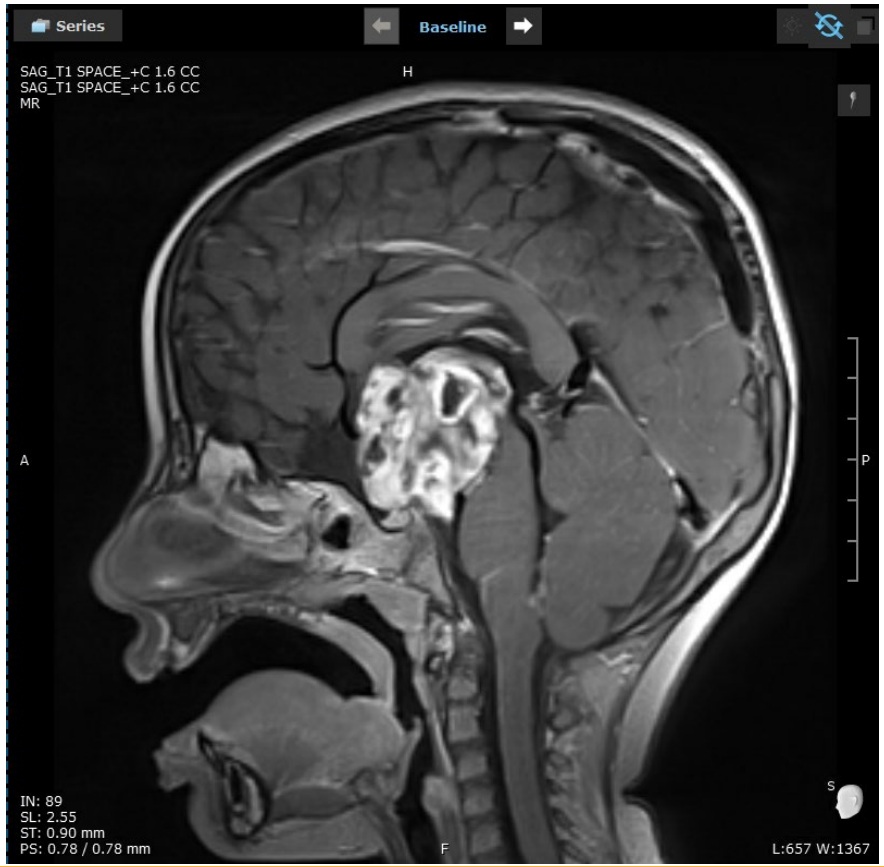
- Investigational, oral, CNS-penetrant Type II RAF inhibitor
- Being developed as tablets and pediatric-friendly liquid suspension
- Breakthrough Therapy Designation
- Rare Pediatric Disease Designation
- Orphan Drug Designation (US/EU)

Growing Portfolio and Runway Beyond Clinical Milestones

- Two clinical-stage MEKi assets, in-licensed for combination trials
- Projected cash runway into 2025[1]
- Key FIREFLY-1 milestones
  - Pre-NDA meeting held on April 19, 2023
  - Initiated rolling NDA submission in May 2023[2]
  - Expected completion of rolling NDA submission in October 2023

[1] With cash, cash equivalents and short-term investments as of March 31, 2023. [2] NDA data set will include analysis of primary (ORR by RANO-HGG) and secondary (ORR by RAPNO-LGG, PFS) efficacy endpoints, safety, and exploratory analyses (including ORR by RANO-LGG).

Pediatric Low-Grade Gliomas (pLGG)



6 y/o with large relapsed *BRAF* fusion-positive optic pathway glioma

Despite being the most common brain tumor in children, there are no approved agents and no standard-of-care for the majority of patients with relapsed/progressive disease[1,2]

Unmet Medical Need

- ~70% of patients will require systemic therapy[1,2]
- Patients have a high rate of recurrence and are frequently treated with multiple lines of systemic therapy over the course of their disease[1,2]
- Many patients today suffer profound tumor and treatment-associated morbidity and significant late effects that persist throughout life[1-4]

Goals of Therapy

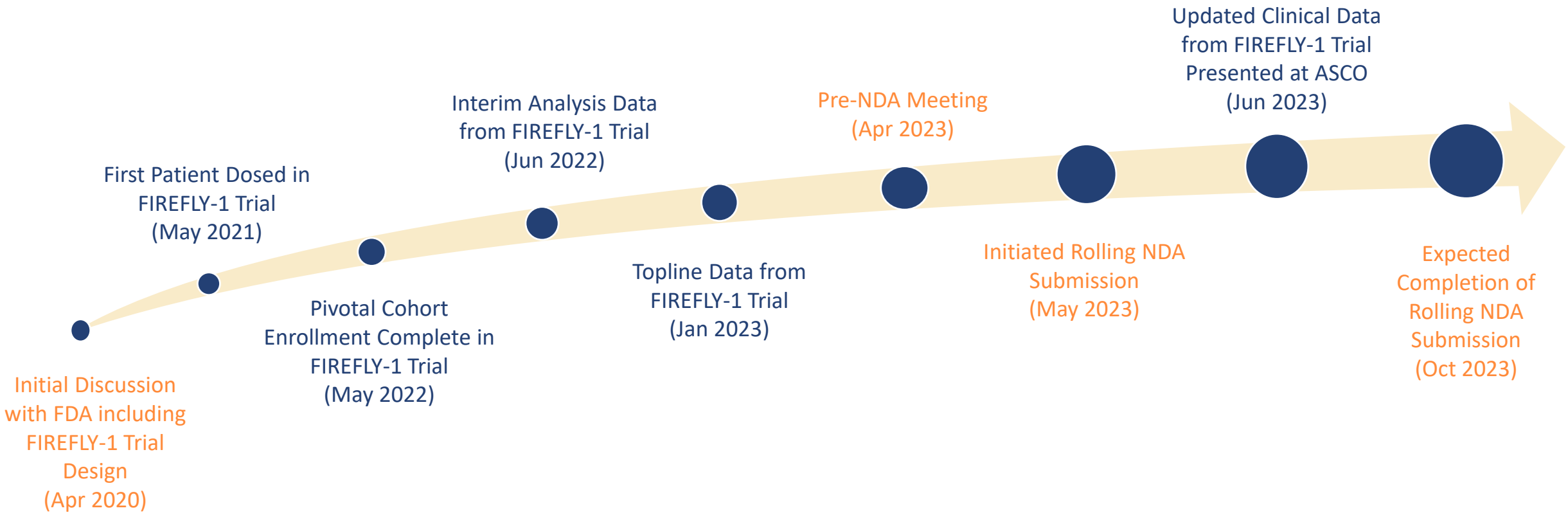
- Stabilize or shrink tumors while minimizing treatment-associated toxicities from surgery, chemotherapy, and radiation

The Majority of pLGGs are Driven by *BRAF* Alterations[3]

- ~85% of *BRAF*-altered tumors harbor a KIAA1549-BRAF gene fusion
- ~15% are driven by *BRAF* V600E mutation

[1] Ostrum QT et al., Neuro Oncol. 2015; 16(Suppl 10):x1-x36; [2] De Blank P. et al., Curr Opin Pediatr. 2019 Feb; 31(1):21-27. [3] Jones DTW et al., Cancer Res. 2008; 68:8673–77. [4] Traunwieser T et al., Neurooncol Adv. 2020; 2:vdaa094.

Progress Of FIREFLY-1 Program: Monotherapy Tovorafenib In Relapsed pLGG



Pivotal Phase 2 Trial Of Monotherapy Tovorafenib (DAY101) In Relapsed Or Progressive pLGG (FIREFLY-1)

Trial Design

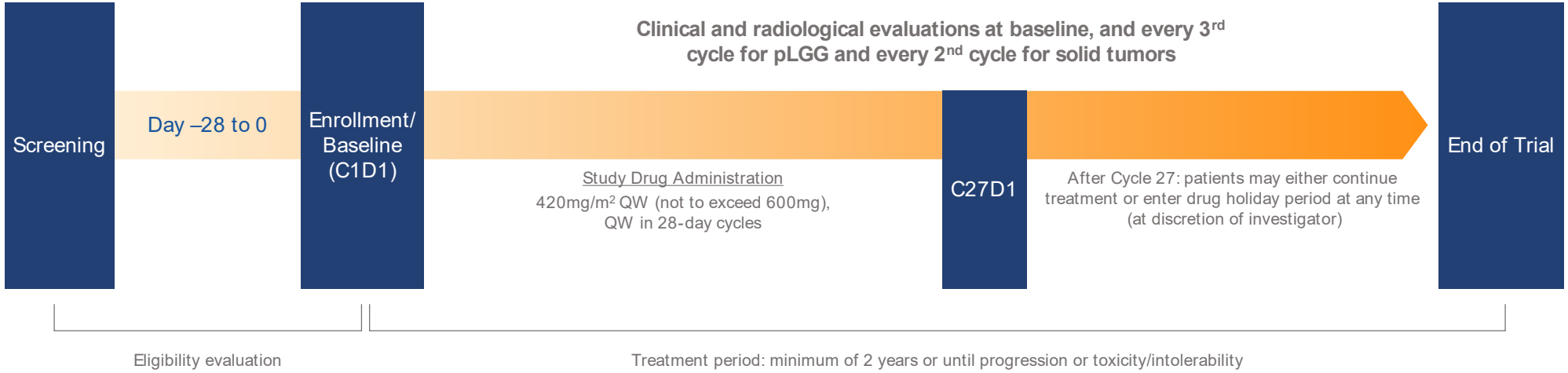
- Three arm, open-label, global registrational phase 2 trial
- **Pivotal Arm 1 (recurrent/progressive pLGG, n=77): harboring a *KIAA1549-BRAF* fusion or *BRAF* V600E mutation**
- Arm 2 (expanded access recurrent/progressive LGG, n=59): harboring an activating *RAF* alteration
- Arm 3 (extracranial solid tumors): harboring an activating *RAF* fusion

Endpoints (Pivotal Arm 1)

- **Primary endpoint: ORR based on RANO-HGG[1], assessed by blinded independent central review**
- Secondary endpoints: ORR by RAPNO-LGG[2] assessed by blinded independent central review; PFS, DoR; TTR, CBR; safety
- Exploratory analyses: ORR and CBR by RANO-LGG[3] assessed by blinded independent central review

Key Inclusion Criteria

- 6 months – 25 years of age
- *RAF*-altered tumor
- ≥1 prior line of systemic therapy with radiographic progression
- Prior use of MAPK pathway targeted therapy was permitted

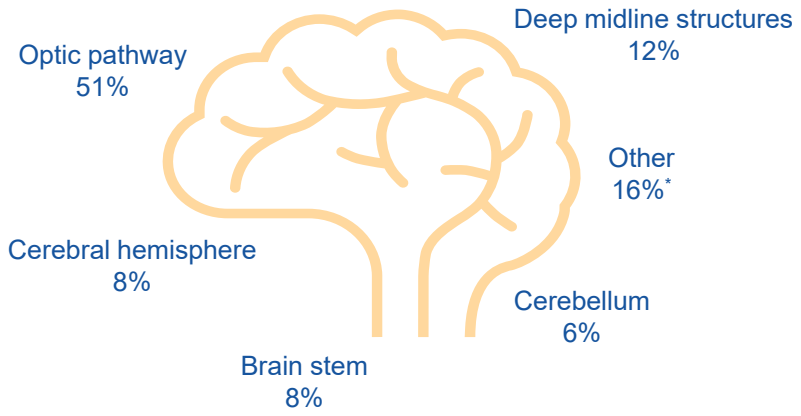


Dec 22, 2022 data cutoff. [1] Wen PY, et al. *J Clin Oncol.* 2010;28(11):1963-1972. [2] Fangusaro J, et al. Lancet Oncol. 2020;21(6):e305–316. [3] van den Bent MJ, et al. Lancet Oncol. 2011;12(6):583-593. Abbreviations: CBR, clinical benefit rate; IRC, independent review committee; C, cycle; D, day; LGG, low-grade glioma; ORR, objective response rate; PFS, progression-free survival; DoR, duration of response; QW, once weekly; TTR, time to response; RANO, Response Assessment in Neuro-Oncology; RAPNO, Response Assessment in Pediatric Neuro-Oncology; MAPK, mitogen-activated protein kinase. For more information, please refer to NCT04775485

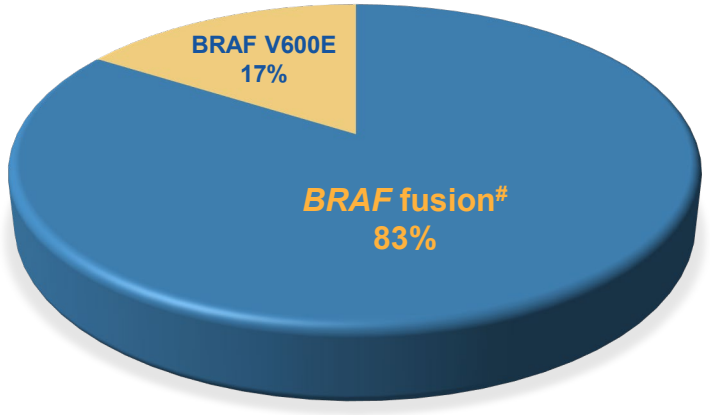
FIREFLY-1 Baseline Patient Characteristics

Characteristic	Arm 1 (n=77)
Median age, years (range)	8 (2-21)
Sex, n (%)	
Male	40 (52)
Female	37 (48)
Race, n (%)	
Black or African American	2 (3)
Asian	5 (6)
White	41 (53)
Multiple	3 (4)
Other	6 (8)
Not reported	20 (26)
Number of lines of prior systemic therapy	
Median (range)	2 (1-9)
1, n (%)	18 (23)
2, n (%)	21 (27)
≥3, n (%)	38 (49)
Prior MAPK pathway targeted therapy, n (%)	46 (60)

Location (n=77)

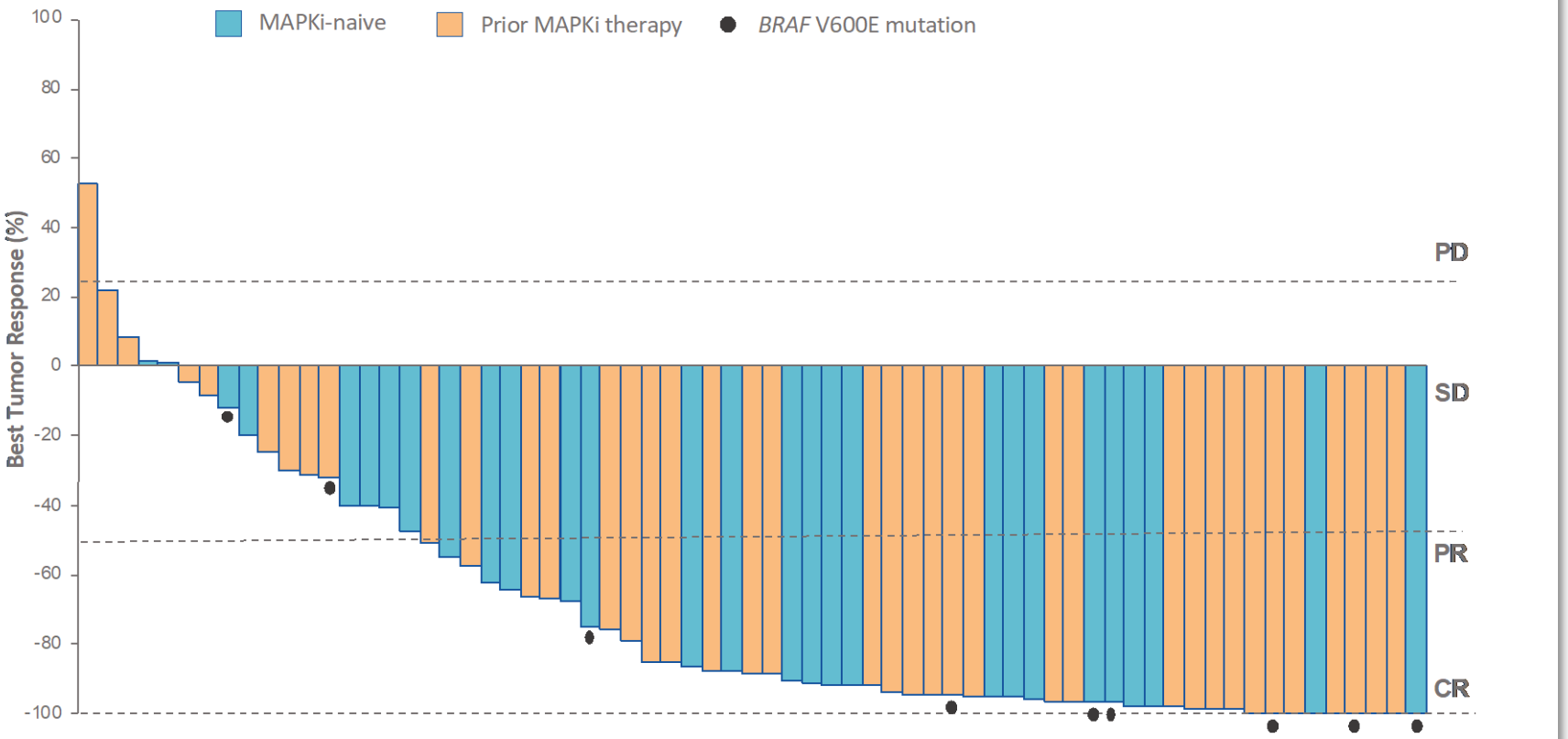


***BRAF* alteration (n=77)**



Dec 22, 2022 data cutoff. *Includes tumors that were extending into multiple regions of the brain, leptomeningeal disease, and/or spinal disease. #Includes 6 patients with *BRAF* duplication and 2 with *BRAF* rearrangement per FISH (Fluorescence in situ hybridization) or ISH (in situ hybridization). MAPK, mitogen-activated protein kinase.

Tumor Response To Tovorafenib (DAY101) For All Patients W ith RANO-HGG Evaluable Lesions (n=69)

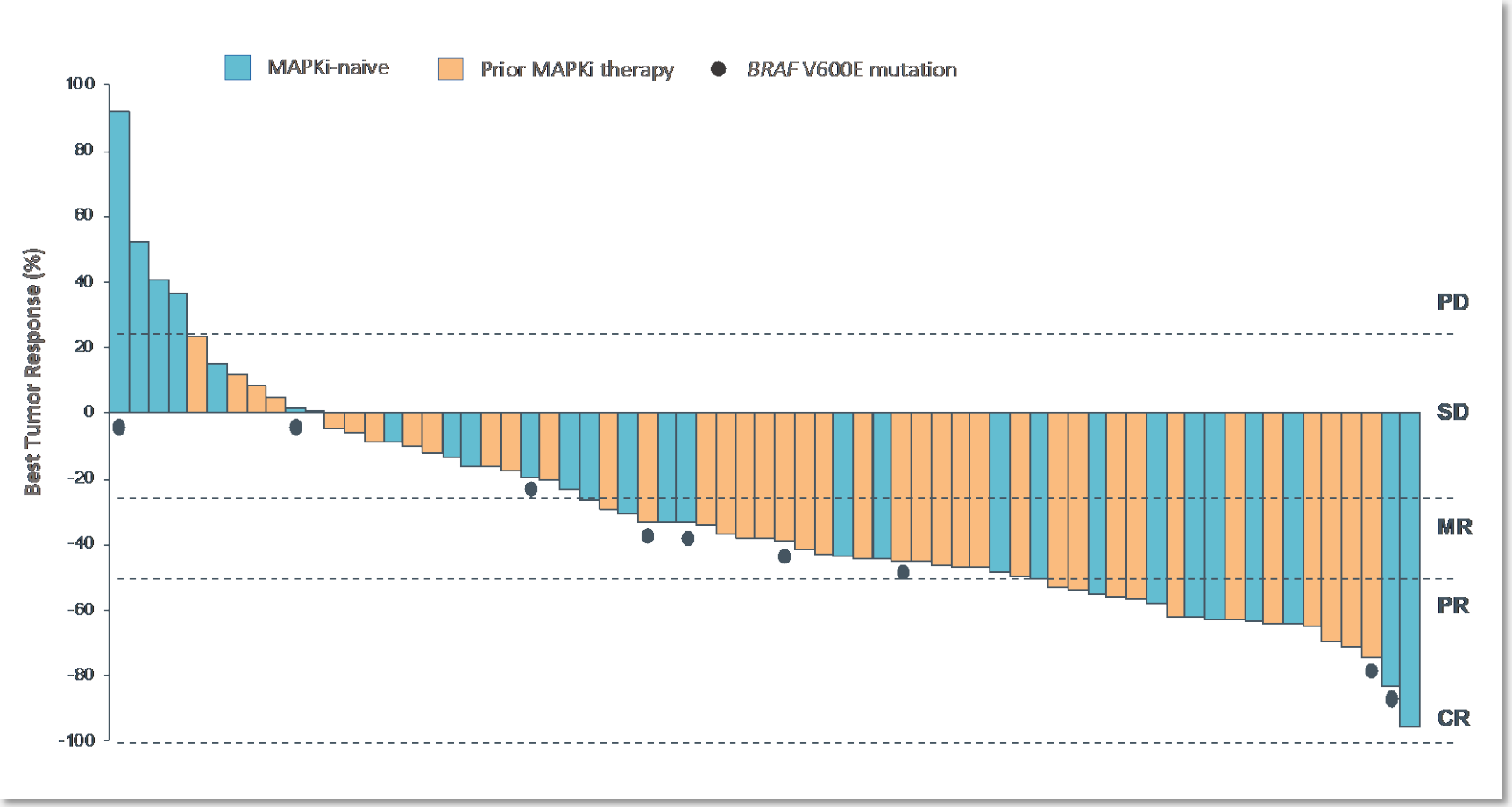


Response (IRC)	RANO-HGG[1] Evaluable n=69
ORR (cCR + cPR + uPR), n (%)	**46 (67%)***
Clinical benefit rate, n (%)	
cCR, cPR/uPR, or SD	64 (93%)
cCR, cPR/uPR, or SD for 12 mo+	47 (68%)
Best overall response, n (%)	
CR	4 (6%)
PR (includes 3 uPR)	42 (61%)
SD	18 (26%)
PD	4 (6%)
Not evaluable	1 (1%)

All 3 patients with uPR remain on treatment as of May 23, 2023

Dec 22, 2022 data cutoff. Percents may not add to 100% due to rounding. Two of 69 patients are not shown in the waterfall plot; one patient passed away due to progressive disease (not related to tovorafenib) before the first imaging assessment and one did not receive T1 Gd+ follow-up imaging. **P*<0.001 from two-sided exact binomial test to test null hypothesis of ORR=21% based on Bouffet et al.[2] [1] Wen PY, et al. J Clin Oncol. 2010;28(11):1963-1972. [2] Bouffet E, et al. J Clin Oncol. 2012;30(12):1358-1363. CBR, clinical benefit rate; cCR, confirmed completed response; cPR, confirmed partial response; CR, complete response; HGG, high-grade glioma; IRC, independent radiology review committee; MAPKi, mitogen-activated protein kinase inhibitor; MR, minor response; ORR, overall response rate; PD, progressive disease; PR, partial response; RANO, Response Assessment in Neuro-Oncology; SD, stable disease; uPR, unconfirmed partial response. There are 17 patients with stable disease of less than 12 months duration and at the time of the data cutoff, 11 remain on treatment.

Tumor Response To Tovorafenib (DAY101) For All Patients With RAPNO-LGG Evaluable Lesions (n=69*)



Response (IRC)	RAPNO-LGG[1] Evaluable n=69
ORR (cCR + cPR/uPR + cMR/uMR), n (%)	**35 (51%)**
Clinical benefit rate, n (%)	
cCR, cPR/uPR, cMR/uMR, or SD	60 (87%)
cCR, cPR/uPR, cMR/uMR, or SD for 12mo+	32 (46%)
Best overall response, n (%)	
CR	0 (0%)
PR (includes 4 uPR)	17 (25%)
MR (includes 4 uMR)	18 (26%)
SD	25 (36%)
PD[#]	8 (12%)
Not evaluable	1 (1%)

All 4 patients with uPR and 3 patients with uMR remain on treatment as of May 23, 2023

Dec 22, 2022 data cutoff. Percents may not add to 100% due to rounding. Two of 69 patients not shown in waterfall plot; one patient passed away due to progressive disease (not related to tovorafenib) before the first imaging assessment and one patient had visual progressive disease but no evaluable T2 measurements at the time of progression. *Pending adjudication. [1] Fangusaro J, et al. Lancet Oncol. 2020;21(6):e305-316. [#]PD for RAPNO-LGG was not used to determine treatment discontinuation; patients could continue treatment if there was no PD based on RANO-HGG per investigator's assessment. CBR, clinical benefit rate; cCR, confirmed completed response; cMR, confirmed minor response; cPR, confirmed partial response; CR, complete response; HGG, high-grade glioma; IRC, independent radiology review committee; LGG, low-grade glioma; MAPKi, mitogen-activated protein kinase inhibitor; MR, minor response; ORR, overall response rate; PD, progressive disease; PR, partial response; RANO, Response Assessment in Neuro-Oncology; RAPNO, Response Assessment in Pediatric Neuro-Oncology; SD, stable disease; uMR, unconfirmed minor response; uPR, unconfirmed partial response. There are 28 patients with stable disease of less than 12 months duration and at the time of the data cutoff, 11 remain on treatment.

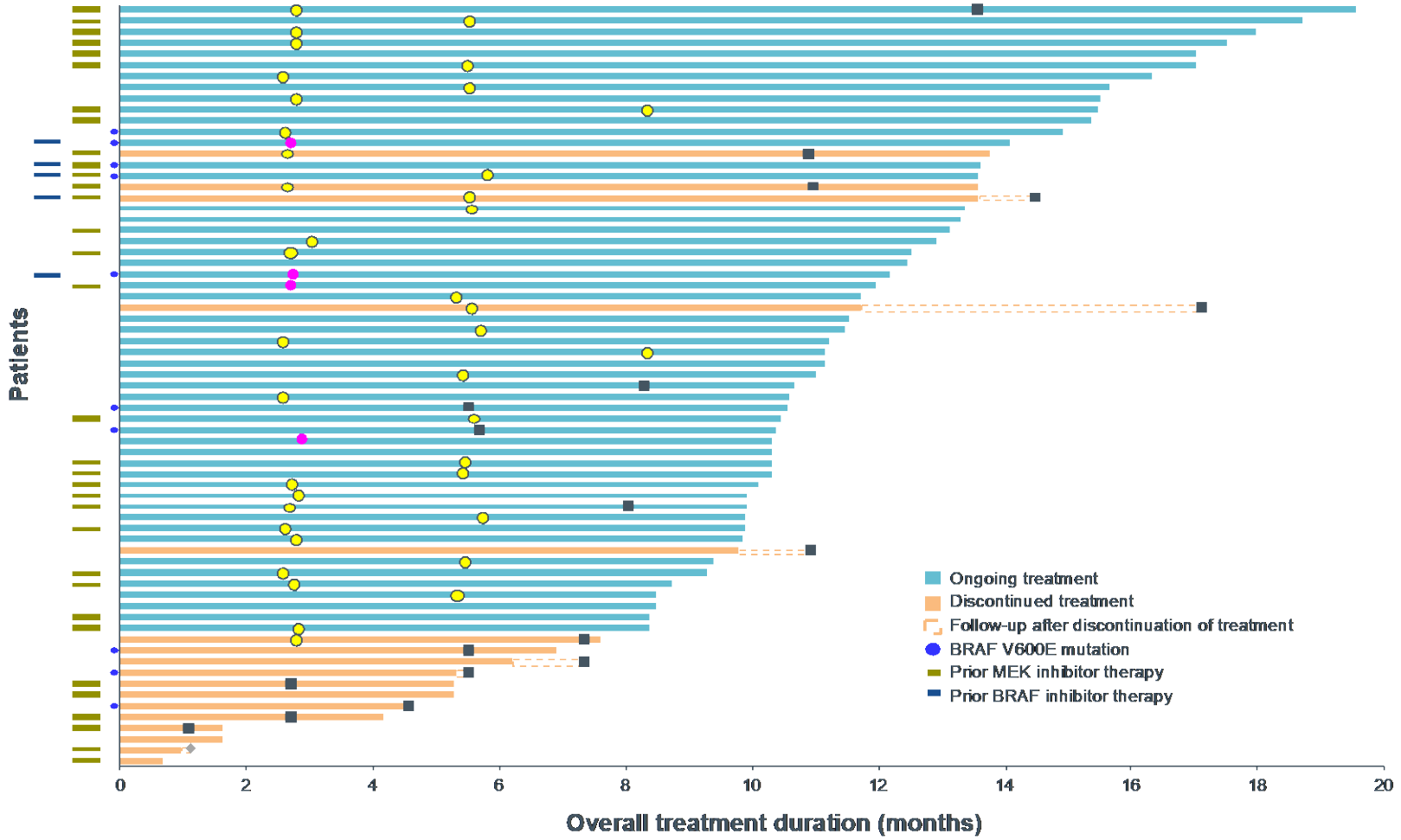
Tumor Response To Tovorafenib (DAY101) For All Patients With RANO-LGG Evaluable Lesions (n=76)

Response (IRC)	RANO-LGG[1] Evaluable n=76
ORR (cCR + cPR/uPR + cMR/uMR), n (%)	**37 (49%)**
Clinical benefit rate, n (%)	
cCR, cPR/uPR, cMR/uMR, or SD	63 (83%)
cCR, cPR/uPR, cMR/uMR, or SD for 12mo+	36 (47%)
Best overall response, n (%)	
CR	0 (0%)
PR (includes 8 uPR)	20 (26%)
MR (includes 2 uMR)	17 (22%)
SD	26 (34%)
PD[#]	11 (14%)
Not evaluable[§]	2 (3%)

All 8 patients with uPR and 2 patients with uMR remain on treatment as of May 23, 2023

Dec 22, 2022 data cutoff. Percents may not add to 100% due to rounding. [#]PD for RANO-LGG was not used to determine treatment discontinuation; patients could continue treatment if there was no PD based on RANO-HGG per investigator's assessment. [§]Two of 76 patients are not shown in the waterfall plot; one patient passed away due to progressive disease (not related to tovorafenib) before the first imaging assessment, and one patient with missing T1 Gd+ imaging at baseline was deemed NE at all timepoints but had a best SPPD decrease of 65% on T2 imaging. [1] van den Bent MJ, et al. *Lancet Oncol*. 2011;12(6):583-593. BL, baseline; CBR, clinical benefit rate; cCR, confirmed completed response; cMR, confirmed minor response; cPR, confirmed partial response; CR, complete response; HGG, high-grade glioma; IRC, independent radiology review committee; LGG, low-grade glioma; MAPKi, mitogen-activated protein kinase inhibitor; MR, minor response; ORR, overall response rate; PD, progressive disease; PR, partial response; RANO, Response Assessment in Neuro-Oncology; SD, stable disease; SPPD, sum of the products of perpendicular diameters; uMR, unconfirmed minor response; uPR, unconfirmed partial response. There are 27 patients with stable disease of less than 12 months duration and at the time of the data cutoff, 19 remain on treatment.

Duration Of Tovorafenib (DAY101) Therapy For All Patients W ith RANO-HGG Evaluable Lesions (n=69)



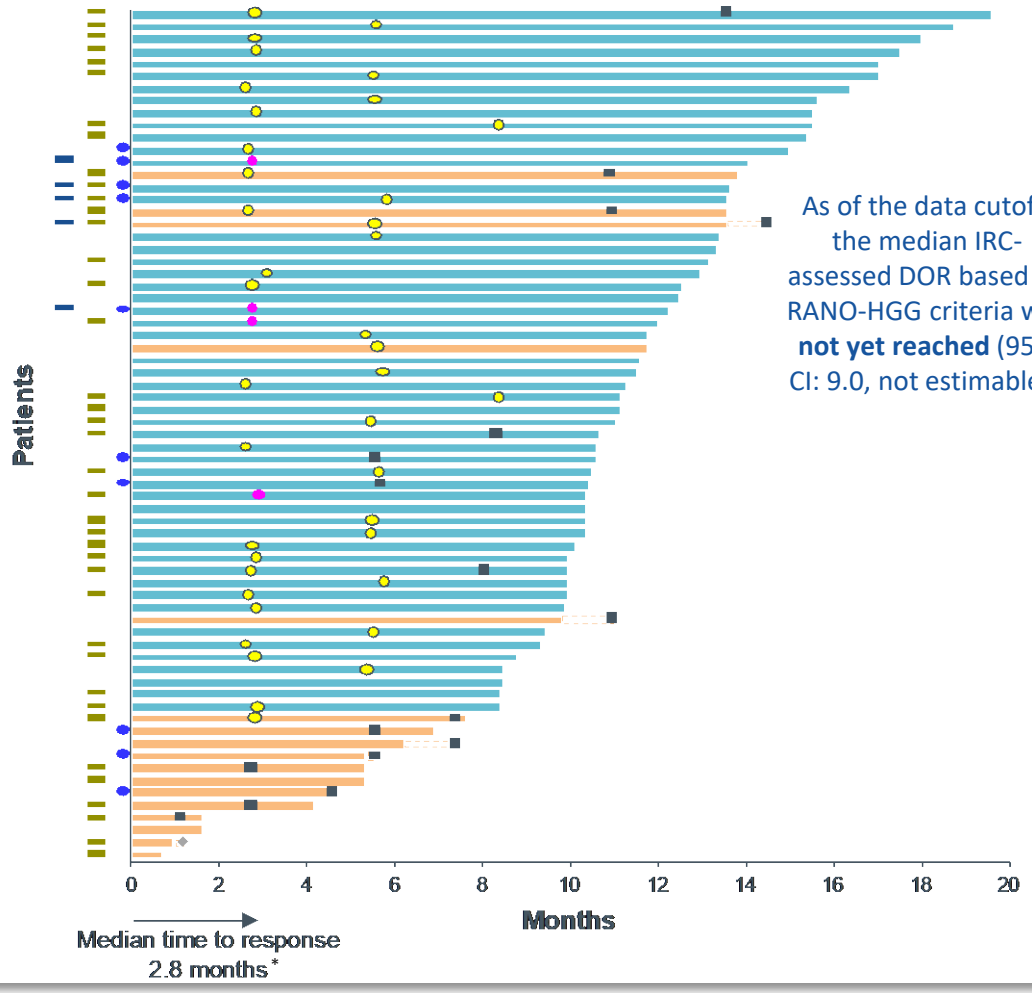
2.8 MONTHS Median time to response*

10.8 MONTHS Median duration of treatment*

* Analysis for median time to response and median duration of treatment only included confirmed responses. HGG, high-grade glioma; RANO, Response Assessment in Neuro-Oncology.

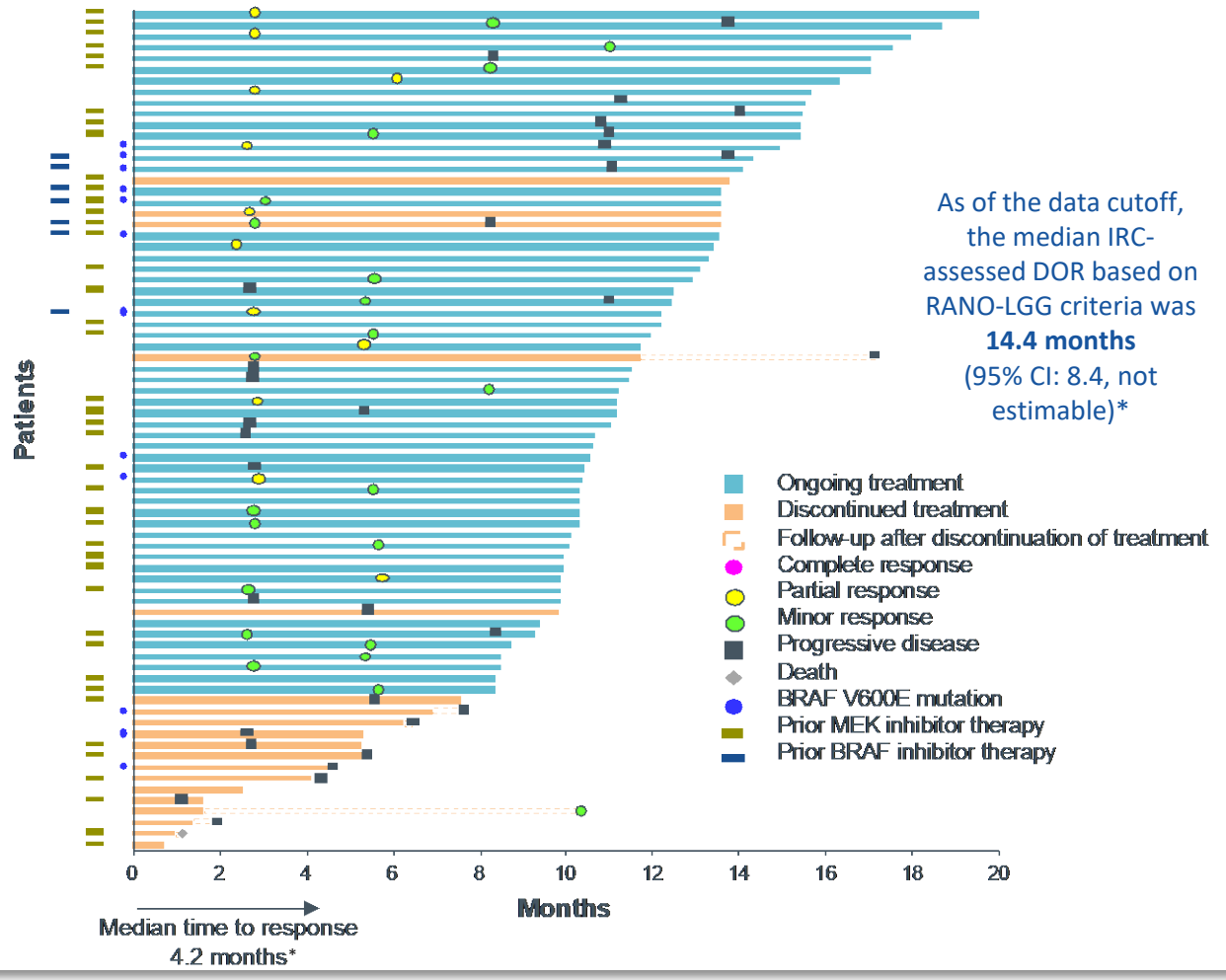
Duration Of Tovorafenib (DAY101) Therapy For All Patients With RANO-HGG & RANO-LGG Evaluable Lesions

RANO-HGG (n=69)



As of the data cutoff, the median IRC-assessed DOR based on RANO-HGG criteria was **not yet reached** (95% CI: 9.0, not estimable)*

RANO-LGG (n=76)

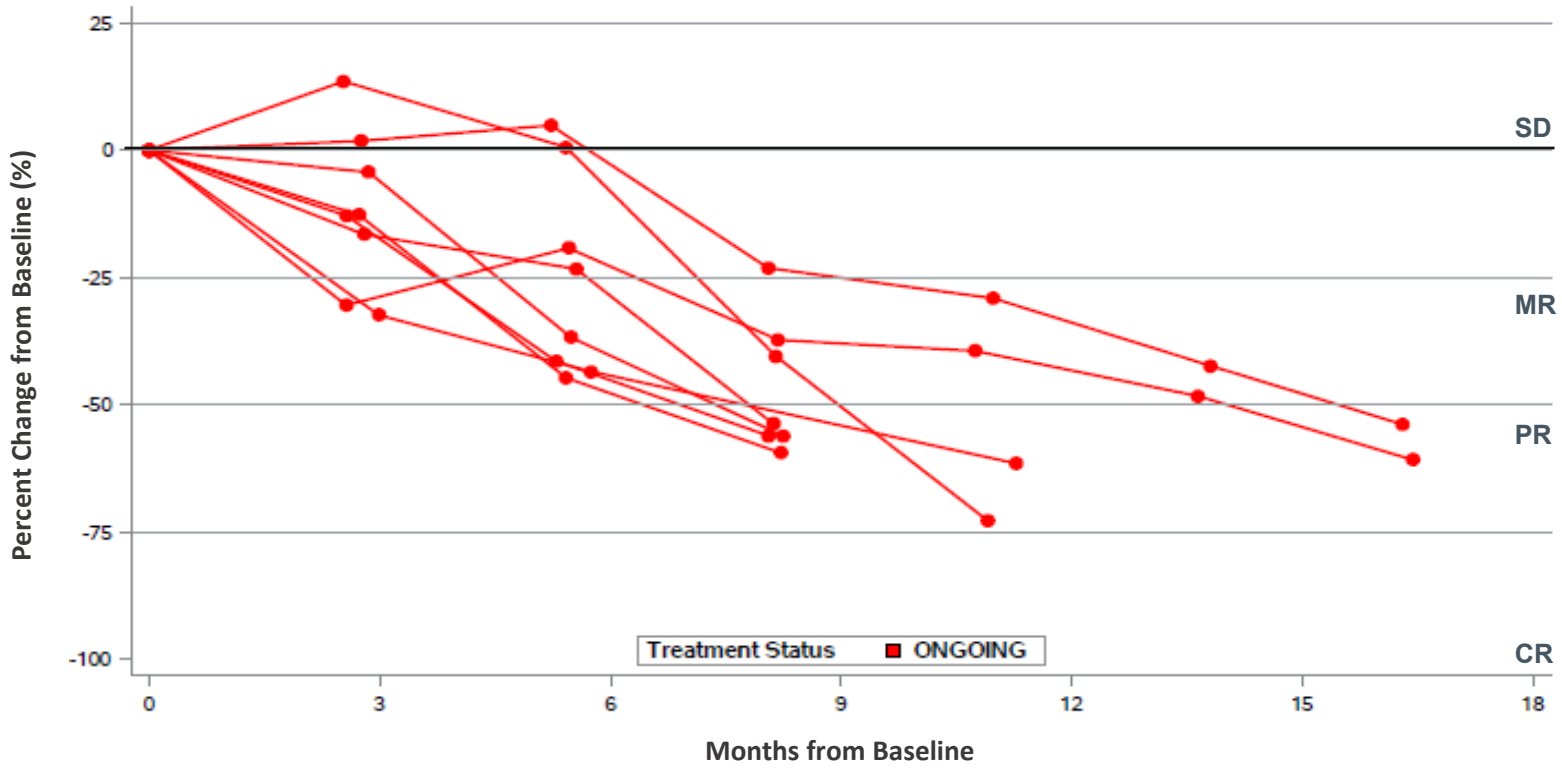


As of the data cutoff, the median IRC-assessed DOR based on RANO-LGG criteria was **14.4 months** (95% CI: 8.4, not estimable)*

* Analysis for median time to response and median duration of response only included confirmed responses. BOR is shown; circles indicate start of response. PD for the purpose of treatment was based on RANO-HGG, not RANO-LGG. BOR, best overall response; HGG, high-grade glioma; LGG, low-grade glioma; PD, progressive disease; RANO, Response Assessment in Neuro-Oncology; DOR, duration of response; CI, confidence interval.

All RANO-LGG Unconfirmed PR Patients Continue On Treatment W ith Demonstrable Deepening Of Response (n=8)

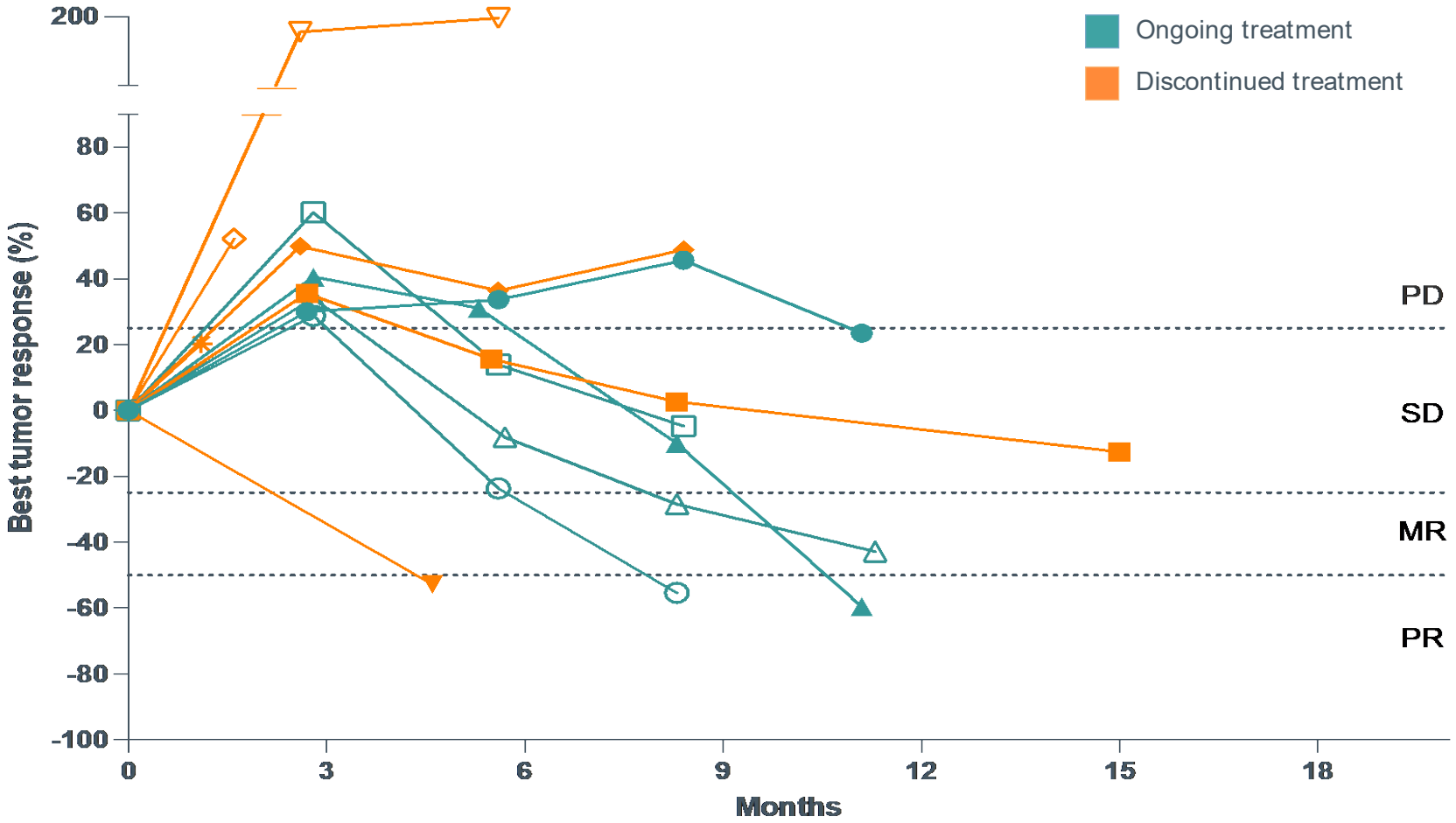
Responses for Patients with Unconfirmed Partial Response by RANO-LGG



All 8 patients with unconfirmed partial response by RANO-LGG remain on treatment as of May 23, 2023

Spider plot for SPPD – baseline and after treatment (RANO-LGG by IRC) – unconfirmed PR patients EOT status based on May 23, 2023 EDC data. Individual patient response data is current as of the data cutoff of December 22, 2022; treatment status data is current as of May 23, 2023.

Nearly Half Of Patients With Best Response Of PD By RANO-LGG Have Tumor Stabilization And Response With Continued Treatment (n=11)



5/11 patients with best response of PD by RANO-LGG remain on treatment as of May 23, 2023

Individual patient response data is current as of the data cutoff of December 22, 2022; treatment status data is current as of May 23, 2023.

Tovorafenib (DAY101) Safety Data (n=136)

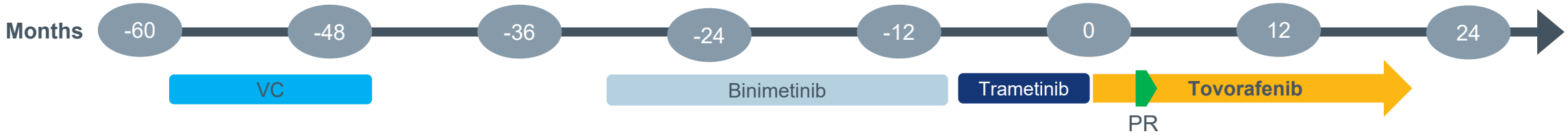
Preferred term, n (%)	Treatment-emergent AEs		Treatment-related AEs	
	Any grade	Grade ≥3	Any grade	Grade ≥3
Any AE	136 (100)	68 (50)	133 (98)	47 (35)
Hair color changes	96 (71)	-	96 (71)	-
Fatigue	68 (50)	4 (3)	54 (40)	4 (3)
Vomiting	59 (43)	3 (2)	24 (18)	3 (2)
Rash maculo-papular	56 (41)	10 (7)	51 (38)	10 (7)
Headache	53 (39)	1 (1)	27 (20)	-
Pyrexia	43 (32)	2 (1)	15 (11)	1 (1)
Nausea	40 (29)	-	21 (15)	-
Dry skin	39 (29)	-	34 (25)	-
Dermatitis acneiform	37 (27)	1 (1)	36 (26)	1 (1)
Constipation	36 (26)	-	28 (21)	-
Decreased appetite	35 (26)	4 (3)	25 (18)	3 (2)
Epistaxis	34 (25)	-	22 (16)	-

- The vast majority of treatment-emergent AEs were Grade 1 or 2
- 39 patients (29%) required dose modifications due to treatment-related AEs
  - Dose interruptions were brief, with the median time of dose interruption being 2 weeks
- 5 patients (4%)* discontinued due to AE, with 4 patients (3%) discontinuing due to treatment-related AEs
- The most commonly reported lab abnormalities were CPK elevation, anemia, hypophosphatemia, and AST elevation
  - Nearly all had no clinical manifestations and did not require clinical intervention or change in study treatment

Dec 22, 2022 data cutoff. Table shows treatment-emergent AEs with frequency ≥25% of any grade. Rash erythematous treatment-emergent: any grade, 14 (10%); grade ≥3 1 (1%); treatment-related: any grade, 14 (10%), grade ≥3 1 (1%). *One patient had 2 events (shunt malfunction [not related to tovorafenib] and tumor hemorrhage [related to tovorafenib]). AEs, adverse events.

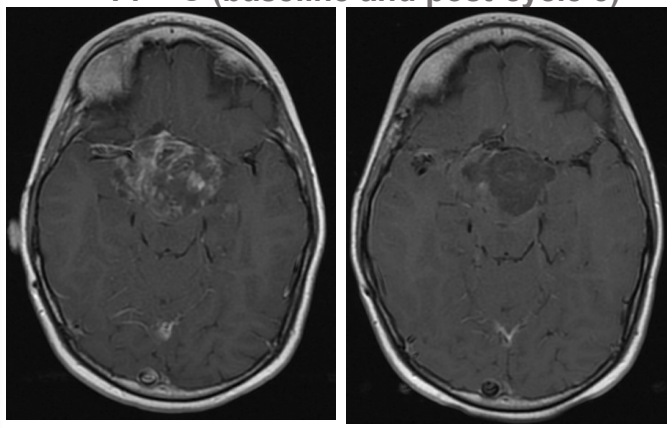
Case Study: Activity Of Tovorafenib (DAY101) In *KIAA1549-BRAF* Fusion Optic Pathway Glioma

8-year-old boy with relapsed pilomyxoid astrocytoma of the optic pathway, with visual loss in right eye, visual field loss in left eye, fatigue, intermittent nausea/vomiting, intermittent headaches, anorexia, and temperature regulation disorder

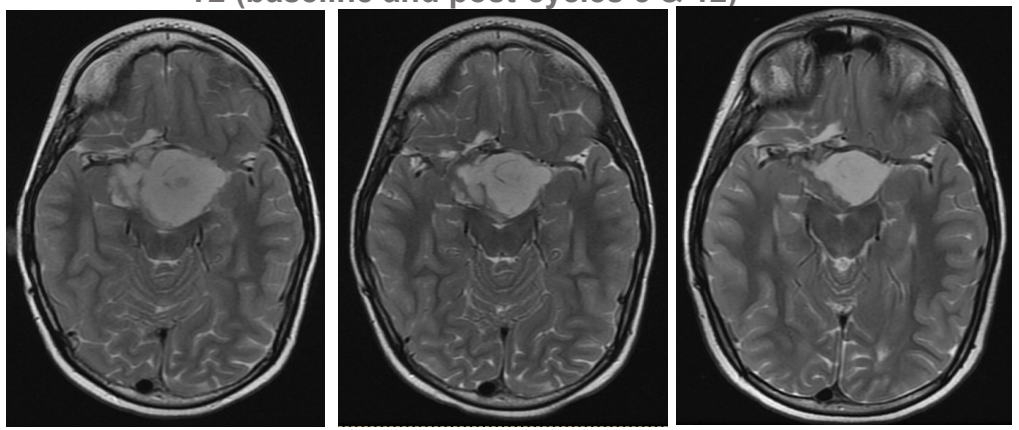


- Initiated treatment with tovorafenib 400 mg/QW following 3 prior therapies, including binimetinib and trametinib, which were discontinued due to PD
- At cycle 3, PR (-88%) per RANO-HGG, and MR (-32% and -40%) per RAPNO-LGG and RANO-LGG, respectively
  - Sustained improvements in visual acuity reported; logMAR change 0.2 → 0
  - PD criteria met (-94% to -91%) with RANO-HGG at cycle 15; continued treatment as investigator deemed no radiographic progression with subsequent reduction in target lesion (-97%)
- AEs were G2 (drug eruption, elevated CPK) and G1 (hair color change, paronychia, growth retardation)

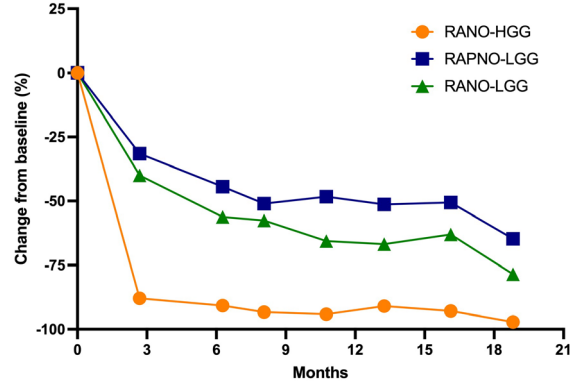
T1 + C (baseline and post-cycle 3)



T2 (baseline and post-cycles 3 & 12)



Tumor kinetics



Dec 22, 2022, data cut-off. AEs, adverse events; C, contrast; CPK, creatine phosphokinase; G, grade; HGG, high-grade glioma; LGG, low-grade glioma; logMAR, Logarithm of the Minimum Angle of Resolution; MR, minor response; PD, progressive disease; PR, partial response; QW, once weekly; RANO, Response Assessment in Neuro-Oncology; RAPNO, Response Assessment in Pediatric Neuro-Oncology; VC, vincristine-carboplatin.

Key Takeaways And Next Steps

- Clinically meaningful data from FIREFLY-1 for pediatric patients with recurrent or progressive LGG harboring *BRAF* fusions or *BRAF* V600E mutations, for whom there is no standard-of-care and no approved agents for the majority of patients
  - 67% ORR and 93% clinical benefit rate (complete response + partial response/unconfirmed partial response + stable disease) by RANO-HGG
  - 51% ORR and 87% clinical benefit rate (partial response/unconfirmed partial response + minor response/unconfirmed minor response + stable disease) by RAPNO-LGG*
    - 8 unconfirmed RAPNO-LGG responses (4uPR, 4uMR) with 7 continuing on treatment as of May 23, 2023
  - 49% ORR and 83% clinical benefit rate (partial response/unconfirmed partial response + minor response/unconfirmed minor response + stable disease) by RANO-LGG
    - 10 unconfirmed RANO-LGG responses (8uPR, 2uMR) with all 10 patients continuing on treatment as of May 23, 2023
- Responses were observed in patients with both *BRAF* fusion and *BRAF* V600E mutations, as well as those who received prior MAPK-targeted therapy
- Rapid time to response regardless of response assessment criteria (median times: 2.8 months with RANO-HGG, 5.5 months with RAPNO-LGG*, and 4.2 months with RANO-LGG)[#]
- A heavily-pretreated population, with a median of 2 prior lines of therapy (range: 1-9), and the majority of patients having relapsed or progressed after one or more prior MAPK inhibitors
- Encouraging safety and tolerability profile indicating monotherapy tovorafenib to be generally well-tolerated
- **Initiated rolling submission of NDA in May 2023**
- FIREFLY-2 Pivotal Phase 3 trial in front-line pLGG is enrolling; first patient dosed in March 2023

Dec 22, 2022 data cutoff. *Pending adjudication. [#] Analysis for median time to response only included confirmed responses.

Day One
BIOPHARMACEUTICALS

Thank you to the patients, their families, and the site investigators and staff who have partnered with us on this study. Together, we remain committed to redefining what is possible for children living with cancer, from Day One and every day after.