PREVALENCE AND IMPACT OF BASELINE NS5A RAVS ON THE EFFICACY OF ELBASVIR/GRAZOPREVIR (EBR/GZR) AGAINST GT1A INFECTION

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BACKGROUND

- **The transition of NS5A RAV testing from research use to the clinic has been complicated by:**
  - a lack of standardization in testing and reporting of individual samples and studies, leading to 'apples to oranges comparisons' in publications and publicly available guidance documents
  - a lack of understanding of the impact of methodology on the interpretation of RAV test results among HCV treaters
- **The magnitude of the potential impact of baseline NS5A RAVs on efficacy depends on:**
  - The method used to detect RAVs
  - The NS5A substitutions that are considered RAVs
  - The duration of therapy and whether ribavirin (RBV) is included
  - The patient population and viral genotype/sub-genotype
- **We evaluated the effect of methodology and RAV definition on the observed impact of baseline NS5A RAVs on the efficacy of elbasvir/grazoprevir (EBR/GZR) among GT1-infected patients**
  - Two detection assays were used: **Population sequencing (~25% ST)** and **next generation sequencing (NGS) at 1% ST**
  - Two definitions of RAVs were used: **EBR RAVs** (specific variants identified in pre-clinical or clinical trials of EBR) and **NS5A Class RAVs** (variants at any position associated with resistance to any NS5A inhibitor)

TWO CLASSES OF GT1A RAVS

Elbasvir (50 mg) | Grazoprevir (100 mg)

NS5A RAV Analysis

- **NS5A Class RAVs (K24*, M28*, Q30*, L31*, P32*, S38*, H58*, A92*, Y93*)**
- **A subset of NS5A Class RAVs that met one or more of these criteria were termed "EBR RAVs"**
  - Identified in *in vitro* replicon selection for resistance assays
  - Recombinant replicons with RAVs observed for other NS5A inhibitors causing *in vitro* potency shifts > 5X for EBR
  - Identified as treatment-emergent RAVs in Phase 1b monotherapy studies
  - Treatment-emergent in virologic failures or in Phase 2 or 3

Group	Position								
	K24	M28	Q30	L31	P32	S38	H58	A92	Y93
EBR RAVs	-¶	A, G, T	D, E, H, G K, L, R	F, M, V	-¶	-¶	D	-¶	C, H, N, S
NS5A Class RAVs	Any	Any	Any	Any	Any	Any	Any	Any	Any

¶No EBR RAVs identified at this position

ANALYSIS POPULATION

NS5A RAV Analysis

- **Populations Evaluated**
  - GT1a-infected patients in Phase 2/3 Program (C-WORTHy, C-EDGE TN, C-EDGE CO-IFXN, C-EDGE TE, C-SURFER)
    - Treatment-Naïve (TN) or Treatment-Experienced (TE) patients who had failed prior therapy with pegylated interferon and ribavirin (PR)
    - Included patients with cirrhosis, chronic kidney disease, and/or HIV co-infection
- **Treatment Regimens—focus on optimal regimens for specific sub-populations†**
  - EBR/GZR for 12 weeks (no ribavirin) for treatment-naïve and prior relapse patients
  - EBR/GZR for 16/18 weeks (with ribavirin) for prior PR non-responders
- **Analyses Conducted in the Resistance Analysis Population (RAP)**
  - Includes 960/986 (97.4%) of the patients in the Full Analysis Set (FAS) of the studies
  - Excludes 21/986 (2.1%) patients who did not achieve SVR for reasons other than virologic failure (loss-to-follow-up; withdrawal from study, etc.)
  - Excludes 5/986 (0.5%) patients for whom baseline NS5A sequencing data was not available, either due to missing sample or failed amplification
- **Primary endpoint: SVR12**

DIFFERENCES IN SEQUENCING FORMAT AND NGS SENSITIVITY THRESHOLDS (ST) RESULT IN DIFFERENCES IN EFFICACY FINDINGS AMONG PATIENTS WITH BASELINE NS5A RAVS

Elbasvir (50 mg) | Grazoprevir (100 mg)

NS5A RAV Analysis

- NGS at 10% and PopSeq are equally efficient at identifying patients harboring baseline NS5A RAVs that reduce the efficacy of EBR/GZR
- NGS at 1% sensitivity threshold (NGS 1%ST) identifies more patients with RAVs than NGS at 10% sensitivity threshold (NGS 10%ST) or PopSeq
  - The efficacy of EBR/GZR remains high among patients identified as harboring baseline NS5A RAVs at the 1% ST but who are not identified at the 10% ST

Example: Treatment-naïve GT1a-infected patients who received 12 weeks of GZR/EBR (no RBV)

Assay	Observed Prevalence of EBR RAVs	No EBR RAVs SVR12	With EBR RAVs SVR12	% NS5A RAVs associated VFs Identified
Population Sequencing	5.8%	383/392 (97.7%)	14/24 (58.3%)	10/12 (83.3%)
Next Generation Sequencing				
≥10% ST	6.7%	381/389 (98.0%)	17/28 (60.7%)	11/12 (91.7%)
>1 to <10% ST	3.1%	12/13 (92.3%)	12/13 (92.3%)	1/12 (8.3%)
≥1% ST	9.8%	369/376 (98.1%)	29/41 (70.7%)	12/12 (100%)

NS5A L31 RAVS: IMPACT OF THRESHOLD OF DETECTION ON EBR/GZR IN TN PATIENTS*

Elbasvir (50 mg) | Grazoprevir (100 mg)

NS5A RAV Analysis

When L31M RAV is present below the 15-20% sensitivity threshold (unless in combination with another RAV) SVR12 is 100%

Patient	NS5A VARIANT			RELATIVE PERCENTAGE		
	Variant #1	Variant #2	Variant #3	Variant #1	Variant #2	Variant #3
1	L31M	--	--	2.66	0	0
2	L31M	--	--	2.75	0	0
3	L31M	--	--	3.65	0	0
4	L31M	--	--	6.46	0	0
5	L31M	M28V	--	11.88	1.85	0
6	L31M	--	--	14.36	0	0
7	L31M	Q30R	--	15.94	77.82	0
8	L31M	--	--	36.91	0	0
9	L31M	M28V	Q30E	43.68	3.6	4.53
10	L31M	--	--	98.83	0	0
11	L31M	Q30H	--	99.2	15.47	0
12	L31M	L31V	M28T	1.1	99.69	1.02
13	L31V	--	--	99.58	0	0
14	L31V	--	--	99.69	0	0
15	L31V	--	--	99.71	0	0

SVR Achieved SVR NOT Achieved

*Analysis includes subjects from Phase 3 TN Patients: PN060/PN061

DIFFERENCES IN THE DEFINITION OF RAVS RESULT IN DIFFERENCES IN EFFICACY FINDINGS AMONG PATIENTS WITH BASELINE NS5A RAVS

Elbasvir (50 mg) | Grazoprevir (100 mg)

NS5A RAV Analysis

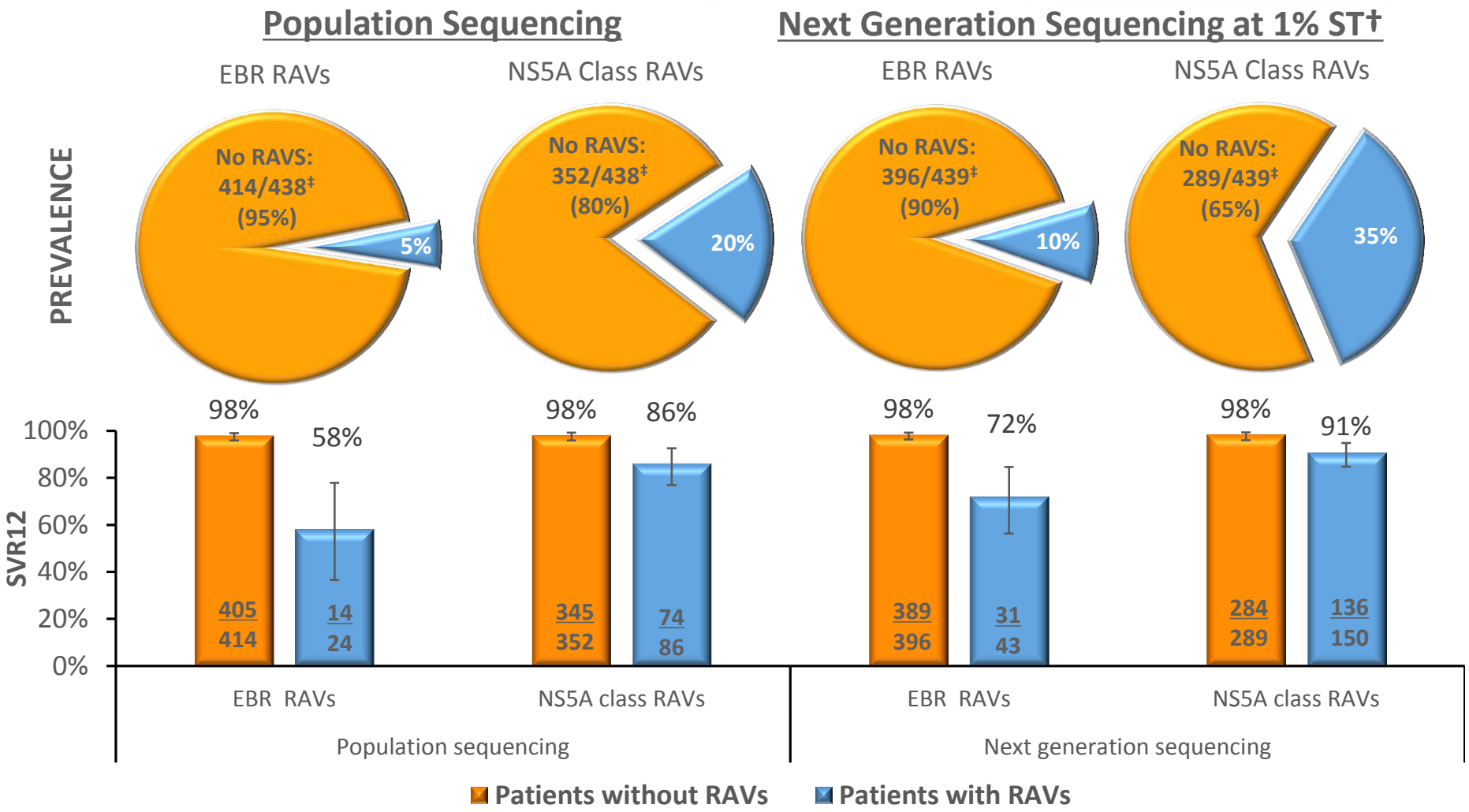
Example: Treatment-naïve GT1a-infected patients who received 12 weeks of GZR/EBR (no RBV)

Assay	EBR RAVs			NS5A Class RAVs		
	Observed EBR RAV Prevalence	SVR12	% NS5A RAVs associated VFs Identified	Observed NS5A Class RAV Prevalence	SVR12	% NS5A RAVs associated VFs Identified
Population Sequencing	5.8%	14/24 (58.3%)	10/12 (83.3%)	20.2%	72/84 (85.7%)	12/14 (85.7%)
NGS (10% ST)	6.7%	17/28 (60.7%)	11/12 (91.7%)	21.8%	78/91 (85.7%)	13/14 (92.9%)
NGS (1% ST)	9.8%	29/41 (70.7%)	12/12 (100%)	35.0%	132/146 (90.4%)	14/14 (100%)

AMONG GT1a TREATMENT-NAÏVE/PRIOR RELAPSERS WITH BASELINE NS5A RAVS, THE EFFICACY OF EBR/GZR (12 WKS, NO RBV) VARIES FROM 58% TO 91%, DEPENDING ON METHODOLOGY

Elbasvir (50 mg) | Grazoprevir (100 mg)

NS5A RAV Analysis



†NGS with 1% ST supplemented by Population Sequencing when NGS not available. ‡ One GT1a was missing baseline population sequencing data but had baseline NGS data

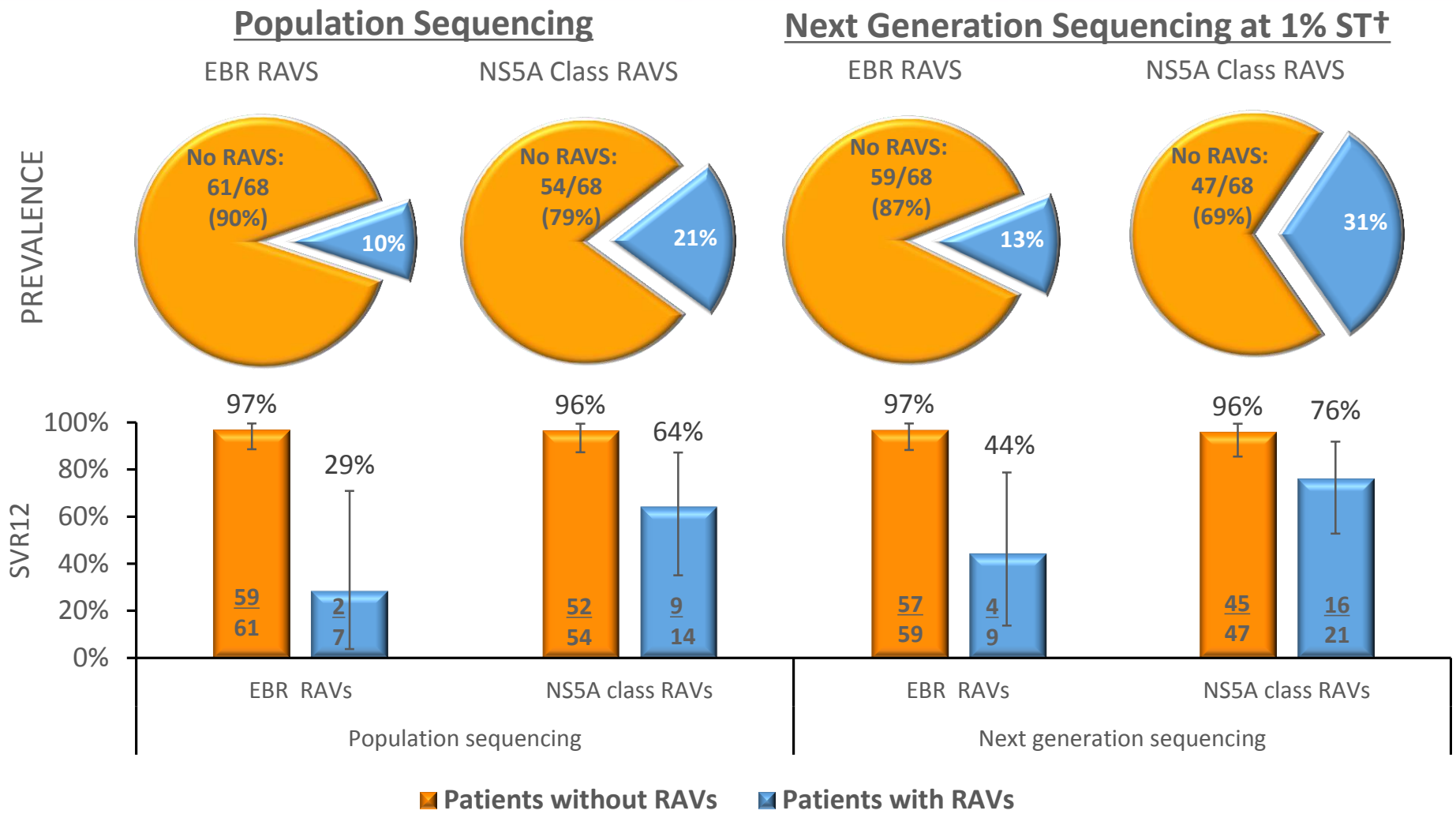
EBR RAV List = For GT1a: M/L28T/A/G, Q/R30E/H/R/G/K/L/D, L31M/V/F, H58D, or Y93C/H/N/S

NS5A Class RAV List = Any variant from reference strain at NS5A position 24, 28, 30, 31, 32, 38, 58, 92 or 93

AMONG GT1a PR NON-RESPONDERS WITH BASELINE NS5A RAVS, THE EFFICACY OF EBR/GZR (12 WKS, NO RBV) VARIES FROM 29% TO 76%, DEPENDING ON METHODOLOGY

Elbasvir (50 mg) | Grazoprevir (100 mg)

NS5A RAV Analysis



†NGS 1% ST supplemented by Population Sequencing when NGS not available. ‡ One GT1a was missing baseline population sequencing data but had baseline NGS data

EBR RAV List = For GT1a: M/L28T/A/G, Q/R30E/H/R/G/K/L/D, L31M/V/F, H58D, or Y93C/H/N/S

NS5A Class RAV List = Any variant from reference strain at NS5A position 24, 28, 30, 31, 32, 38, 58, 92 or 93

EFFECT OF RAVS AT SPECIFIC BASELINE POSITIONS ON LIKELIHOOD TO ACHIEVE SVR12

Elbasvir (50 mg) | Grazoprevir (100 mg)

NS5A RAV Analysis

GT1a-Infected TN/TE Subjects given EBR/GZR 12 weeks (no RBV)

RAV Position	SVR12 Subjects with RAVs (1% ST NGS)	SVR12 Subjects With RAVs (Population Sequencing)
24	15/18 (83.3%)	4/4 (100.0%)
28	61/68 (89.7%)	29/33 (87.9%)
30	14/23 (60.9%)	4/10 (40.0%)
31	15/23 (65.2%)	5/13 (38.5%)
32	1/1 (100.0%)	--
38	9/9 (100.0%)	--
58	75/77 (97.4%)	48/49 (98.0%)
92	6/6 (100.0%)	3/3 (100.0%)
93	9/14 (64.3%)	5/8 (62.5%)

NGS using 1% ST supplemented by Population Sequencing when NGS not available

NS5A Class RAV List = Any variant from reference strain at NS5A position 24, 28, 30, 31, 32, 38, 58, 92 and 93

RELAPSE RATES IN TN/TE(PR) GT1A SUBJECTS BY NUMBER OF BASELINE NS5A RAVS (POPULATION SEQUENCING)

Elbasvir (50 mg) | Grazoprevir (100 mg)

NS5A RAV Analysis

Number of BL RAVs	EBR RAV List			M28*, Q30*, L31*, H58*, Y93*		
	Relapses	All Subjects	Relapse rate	Relapses	All Subjects	Relapse rate
EBR/GZR (No RBV) for 12 Weeks						
1	11	27	41%	11	79	14%
2	3	4	75%	3	4	75%
3	0	0	0%	1	1	100%
Total	14	31	45%	15	84	18%
EBR/GZR + RBV for 16/18 Weeks						
1 RAVs	0	6	0%	0	22	0%
2,3 RAVs	0	1	0%	0	1	0%

SUMMARY AND CONCLUSIONS

NS5A RAV Analysis

- Presence of certain NS5A RAVs at baseline among patients with GT1a infection have been shown to reduce the efficacy of regimens containing NS5A inhibitors
- The magnitude of this lower efficacy is impacted by the methodology used to detect RAVs and the list of RAVs chosen for evaluation. Among GT1a-infected patients given EBR/GZR (no RBV) for 12 weeks:
  1) With population sequencing or 10% ST NGS and a focus on a small set of EBR RAVs, the efficacy of EBR/GZR is reduced in the presence of NS5A RAVs at baseline
  2) With 1% ST NGS and a broad definition of NS5A RAVs, the impact of baseline NS5A RAVs on the efficacy of EBR/GZR is minimal
- Currently, guidance documents regarding the impact of NS5A RAVs report findings with varying methodologies, so it is difficult for practitioners to compare treatment regimens
- Development of standardized methods and definitions will enable a better understanding of how to optimize therapy and assess “resistance”