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Long term follow-up of EGFR mutated NSCLC cases

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ABSTRACT

Purpose: A substantial fraction of all non-small cell lung cancers(NSCLC) carry a mutation in the EGFR gene for which an effective treatment with anti-tyrosine kinases(TKIs) is available. We studied the long term survival of these patients following the introduction of TKIs.

Experimental design: All consecutive cases of NSCLC newly diagnosed with advanced disease were referred for free tumor EGFR mutation testing at Clalit's national personalized medicine laboratory. Mutations and deletions in target codons 18–21 of EGFR were sought using RT-PCR and fragment analysis. Comprehensive EMRs were used to collect full data on treatments and clinical status.

Results: A cohort of 3,062 advanced NSCLC cases, included 481(15.7%) somatic EGFR mutation carriers (17.5% of all adenocarcinomas, 26.7% of females with adenocarcinomas). TKIs treatment to EGFR mutation carriers was provided to 85% of all eligible. After a median follow up period of 15.9 months for EGFR mutated cases the hazard ratio for overall survival of EGFR-mutated NSCLC treated with TKIs was 0.55(0.49–0.63, $p<0.0001$) when compared with EGFR wild-type(WT) tumors under usual care. After adjusting for age, sex, ethnicity, smoking history and tumor histology, all of which had an independently significant effect on survival, the HR for TKI-treated, EGFR-mutated tumors, was 0.63 (0.55–0.71, $p<0.0001$). Treating EGFR-WT cases with TKIs yielded a high HR=1.32 (1.19–1.48).

Conclusions: TKIs given to EGFR mutated advanced NSCLC demonstrated a substantial survival benefit for at least five years. Squamous histology, smoking, male sex and Arab ethnicity were associated with higher NSCLC mortality hazard. Treating non-EGFR-mutated NSCLC with TKIs seems detrimental.

Statement of Significance:

- TKIs given to EGFR mutated advanced NSCLC demonstrated a substantial survival benefit for at least five years but not much longer.
- Treating non-EGFR-mutated NSCLC with TKIs seems detrimental and should probably be avoided.
- Squamous histology of non-small cell lung cancer, smoking history, male sex and Arab ethnicity were associated with altogether higher NSCLC mortality hazard.

Background

Lung cancer is the leading cause of cancer related death in Israel and world-wide [1]. Most of these cases are non-small cell lung cancer (NSCLC). Recent advances in the treatment of this disease include the advent of targeted agents for patients harboring a driver mutation [2–7]. The most common targeted driver gene is the epithelial growth factor receptor (EGFR). The prevalence of activating EGFR mutations among lung cancer patients ranges from 8% in eastern Europe [8] to around 50% in eastern Asia [9]. Most EGFR mutations in NSCLC are either a small deletion in exon 19 or the L858R mutation in exon 21, causing tyrosine kinase to be overactive and mutated tumors to be sen-

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sitive to inhibition by targeted EGFR inhibitors. Patients with advanced NSCLC harboring such sensitizing EGFR mutations benefit from treatment with 1st or 2nd generation EGFR Tyrosine Kinase Inhibitors (TKIs) [3,5,10–12] but resistance always evolves, usually after less than a year of treatment[13]. Apart from one randomized prospective study that demonstrated overall survival (OS) improvement for EGFR mutation positive patients with EGFR-TKI treatment compared to chemotherapy (only within the exon-19 deletion sub-group)[14], all other trials failed to show better OS. A meta-analysis of trials including these patients also failed to show overall survival benefit [15,16]with EGFR-TKI use. However, retrospective analyses of NSCLC demonstrates longer survival for EGFR mutation positive patients treated with EGFR TKIs [17,18]. Most retrospective studies lack validated data about the actual administered treatment regimens, and suffer from various methodological biases.

The most common mechanism of resistance is a gate-keeper mutation in the EGFR gene, T790M, present in about 50% of patients with acquired resistance to 1st and 2nd generation EGFR-TKIs. 3rd generation TKIs have been demonstrated to be the optimal treatment in this scenario [2]. A recent report highlights the possibility of administration of 3rd generation TKI as the first-line treatment for EGFR mutation positive patients [19] and demonstrated advantageous progression free survival (PFS) when compared to first line treatment with 1st or 2nd generation TKIs. Similar to most studies with EGFR-TKIs, no OS benefit was seen. Thus, the choice of first-line treatment of EGFR mutant patients is currently based on PFS data. We report here a full unselected cohort of advanced NSCLC patients, with and without mutations and with and without TKIs treatment, for their real-life data and overall survival.

Methods

All consecutive cases of advanced non-small-cell lung cancer (NSCLC) referred to the National Personalized Medicine Program of Clalit Health Services for molecular tissue analysis in the years 2013–2017 were included in this analysis. Clalit Health Services is the largest health services provider in Israel, covering about 4.5 million people. The Israeli National Health Law services basket enables these tests free of charge for advanced cases and the identification of a mutation in EGFR, ALK or ROS1 is required for the approval to receive one of the relevant proven biological treatments with TKIs. The National Laboratory of Clalit performed the vast majority of these molecular tests in Israel. Data were collected at time of testing regarding age at testing, sex, smoking history, ethnicity (Jews/Arabs), histological type of tumor (Adenocarcinoma /Squamous Cell Carcinoma /other) and the exact type of mutation in EGFR. Updated vital status was available for all cases analyzed.

Treatments

All mutation-targeted medications require approval prior to use. Treatment details (duration, generic drug name) were available from Clalit's comprehensive EMRs. TKIs of interest for this study included: Erlotinib (Tarceva), Gefitinib (Iressa), Afatinib (Gilotrif). Approved medications were provided free of charge.

Molecular tests

EGFR mutation tests included recognized sensitizing mutations in exons 18–21. Several point mutations are routinely tested for, using RT-PCR (exon 18 [codon 719], exon 20 [codon790], exon 21 [codons 858, 861]) or Sanger sequencing (exon 18 [codons 709, 719], exon 19, exon 20 [codon 790 and since 2015 also codon 797], exon 21 [codons 858, 861]). Exon 19 is also routinely studied for insertions or deletions using fragment length analysis. These tests are sensitive enough to detect a genetic modification given sufficient material for analysis. In most cases tests were performed by two independent methods, and identified mutations were always confirmed by a different molecular method.

ALK mutations were detected in only a tiny minority of the NSCLC cases and are not reported in this manuscript. PDL1–associated tests and treatments were not in practice during this study's period. EGFR mutations are identified from tumor specimens from patients with NSCLC using DNA sequencing, RT-PCR or fragment length analysis. Briefly, DNA was extracted from paraffin-embedded tumor samples using a commercially available kit, according to the manufacturer's recommendation (QIAmp DNA mini kit, Qiagen). Genotyping of exons 18, 20, and 21 using SNP Assay-by-Design was performed by allelic discrimination using a Taqman- based SNP genotyping assay on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City CA, USA). The assay was performed in a 20 μl reaction volume containing 1 μl genomic DNA, 0.15 μl primer/probe mix, 5 μl TaqMan genotyping master mix (Applied Biosystems), and 14 μl of double distilled water. The thermocycling set-up includes a pre-run of 2 min at 50 °C, followed by 10 min at 95 °C; then 50 cycles with 10 s at 95 °C, followed by 60 s at 60 °C. Primers and probes were generated by the Assay-by-Design custom oligonucleotide reagent service (Applied Biosystems) and are available upon request. In parallel and independently, all samples were sequenced for exons 18, 19, 20, and 21. Direct sequencing reactions were performed in the ABI 3130 Sequencer. Fragment length analysis isolates the EGFR exon 19 region (a fragment spanning amino acids 700–800) via PCR reaction with the following FAM labeled primers: *Forward 5′ - FAM -GTGCATCGCTGGTAACATCC –3′,Reverse 5′ -TGTGGAGATGAGCAGGGTCT – 3′*.PCR products were diluted 1:10 and 1 μl was added to a reaction solution containing 8.5 μl Formamide and 0.5 μl GeneScanTM – 500 ROX^{TM} Size Standard (Applied Biosystems). Fragment analysis was performed with the 3130xl Genetic Analyzer (Applied Biosystems). Deletions and/or insertions were clearly observed by a change in the fragment size.

Statistical analysis

We compared overall survival of cases with identified sensitizing mutations in EGFR who received treatment with tyrosine-kinase inhibitors (TKIs) with that of cases with mutations who did not receive treatment with TKIs, and with cases without mutations. Cox proportional hazard ratios were used to compare survival of the various groups (with/without mutation, with/without TKI treatment). Overall survival was calculated as time from start of treatment for those receiving TKIs or from date of provision of genetic test results for those who were not treated with TKIs and until death or censored at data cutoff (21/8/2017) and Kaplan Meier curves were Drawn. Multivariate models were employed to control for the effect of other relevant variables on survival. Hazard ratios and 95% confidence intervals are presented. All analyses were done using IBM SPSS version 24.

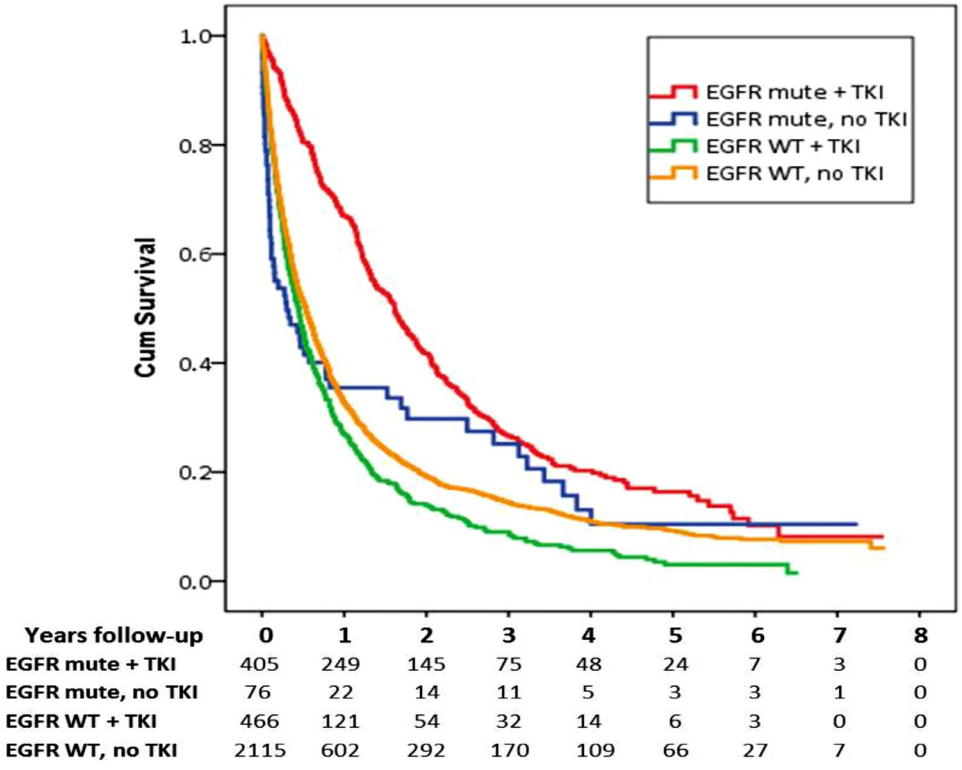
Results

3062 consecutively referred cases of advanced NSCLC were tested during the years 2013–2017 for EGFR in the National Personalized Medicine Laboratory of Clalit Health Services. The median follow-up time was 15.9 months for the EGFR mutated cases who received TKIs, 3.5 months for untreated EGFR mutated cases and 5.2 months for the cases with wild-type EGFR.

Somatic mutations in EGFR were detected in 15.7% of our study group, and involved mainly deletions in exon 19 (54.2%) and the L858R mutation in exon 21 (35.2%). Table 1 describes the demographic and clinical characteristics of the cases included in this analysis. Most cases referred for testing (81%) were adenocarcinomas. Males predominated among cases with EGFR wild-type tumors (64.4%) but women were significantly more common among cases with EGFR mutations, either with adenocarcinomas (63.0%) or squamous cell carcinomas (76.9%). Ever smoking was much more common among cases with wild-type tumors

Table 1
Characteristics of cases with advanced NSCLC referred for molecular analysis at Clalit's National Personalized Medicine Laboratory.

	EGFR Wild Type ($n=2581$)				EGFR With Mutation ($n=481$)			
Histology	Adeno	Squamous	other	unknown	Adeno	Squamous	other	unknown
Number of cases	2045	304	186 (7.2)	46	433	13	33	2
(%)	(79.2)	(11.8)		(1.8)	(90.0)	(2.7)	(6.9)	(0.4)
Age at test	69.1 (11.01)	72.0	68.3	75.2	71.3	76.2 (9.63)	70.4	77.5
(mean + sd)		(9.73)	(10.90)	(9.98)	(11.70)		(12.74)	(10.61)
Sex								
Males (%)	1296 (63.4)	221	117 (62.9)	29	160 (37.0)	3 (23.1)	10	1
		(72.7)		(63.0)			(30.3)	(50.0)
Females (%)	749 (36.6)	83	69 (37.1)	17 (37.0)	273 (63.0)	10 (76.9)	23 (69.7)	1
		(27.3)						(50.0)
Ethnicity								
Jews (%)	87.7	83.9	90.3	97.8	91.0	92.3	97.0	100.0
Non-Jews	12.3	16.1	9.7	2.2	9.0	7.7	3.0	0.0
Smoking								
Ever (%)	75.9	88.2	83.8	82.6	40.2	38.5	57.6	50.0
% TKI treated	19.2	7.6	26.9	0.0	84.8	76.9	84.8	0.0



Years follow-up	0	1	2	3	4	5	6	7	8
EGFR mute + TKI	405	249	145	75	48	24	7	3	0
EGFR mute, no TKI	76	22	14	11	5	3	3	1	0
EGFR WT + TKI	466	121	54	32	14	6	3	0	0
EGFR WT, no TKI	2115	602	292	170	109	66	27	7	0

Fig. 1. Survival of cases of NSCLC, by EGFR status and TKI-treatment status, Clalit Health Services.

(82%), compared to cases with EGFR mutations (38.5%). Treatment with TKIs was administered to 85% of EGFR mutant adenocarcinoma cases and 77% of EGFR mutant squamous cell carcinoma cases and also to almost 20% of adenocarcinoma cases who tested negative to EGFR. The vast majority (85%) of EGFR mutation carriers received TKI treatment within one month from the genetic testing, while the median time to treatment for the EGFR wild type patients was 7 months, with 75% of them receiving treatment more than 3 months after negative mutation testing result.

Lung cancer cases with somatic EGFR mutations who received treatment with TKIs had a clinically meaningful and statistically significant overall survival benefit (HR=0.59, 95% C.I. 0.52–0.66, $p<0.0001$) compared to cases with no somatic mutations in EGFR who received usual care chemotherapy, an effect that lasted for some 6 years and was significant throughout the follow-up period. At a 2-year follow-up point, the survival of TKI treated cases with EGFR mutation was 42% while that of EGFR wildtype with no TKI treatment was only 19% (Fig. 1).

While TKI-treated cases with EGFR mutated tumors showed significantly improved survival, the small group ($n=71$) of cases with mutated EGFR who did not receive TKI treatment showed lower survival rates which were similar to that of the cases with EGFR-WT tumors, HR=1.00 (0.77–1.30, n.s.). Cases with EGFR-WT tumors who did receive treatment with TKIs ($n=466$) had a high hazard ratio from start of treatment to death compared to WT patients who did not receive TKIs (HR=1.22, 1.09–1.35, $P<0.0001$). This group tended to start their TKI treatment several months after the negative molecular test result. Survival of NSCLC patients demonstrated an influence of demographic, behavioral and clinical parameters with worse survival of males, Arabs, smokers and those with squamous cell histology (Table 2). Among mutation positive patients treated with TKIs, men who smoked had a significantly worse outcome compared to male or female non-smokers.

In a multivariate cox proportional hazards model which included the statistically significant confounders age (HR=1.01/year), sex (HR=1.30 for males), ethnicity (HR=0.87 for Jews), smoking history (HR= 1.12

Table 2
Univariate effects (Hazard ratio) of treatment with TKIs by EGFR-mutation, smoking, ethnicity and histology status in cases with advanced NSCLC.

	HR	95% CI -	95% CI +	P
EGFR Wild-Type (WT) - **no** treatment with TKIs	1.00			
EGFR WT - **with** treatment with TKIs	1.22	1.093	1.351	<0.0001
EGFR mutated - **no** treatment with TKIs	1.00	0.771	1.297	
EGFR mutated - with treatment with TKIs	**0.55**	**0.489**	**0.626**	**<0.0001**
Smoking				
EGFR mutated with TKIs - non-smoking females	1.00			
EGFR mutated with TKIs - non-smoking males	0.99	0.703	1.403	.969
EGFR mutated with TKIs - smoking females	1.02	0.745	1.397	.902
EGFR mutated with TKIs - smoking males	1.42	1.063	1.897	.018
Ethnicity				
EGFR mutated with TKIs – Jewish smoking males	1.00			
EGFR mutated with TKIs – Arab smoking males	1.49	0.762	2.927	.243
Histology				
EGFR mutated with TKIs - adenocarcinoma	1.00			
EGFR mutated with TKIs - squamous cell carcinoma	1.76	0.903	3.419	.097

Table 3
Multivariate cox proportional hazards model of TKIs treatment effect by EGFR mutation status in NSCLC cases.

	HR	95% CI -	95% CI +	P
EGFR Wild-Type (WT) without TKIs treatment	1.00			
EGFR mutated with TKIs treatment	**0.63**	**0.549**	**0.712**	**<0.0001**
EGFR mutated without TKIs treatment	1.07	0.813	1.418	0.62
EGFR WT with TKIs treatment	1.32	1.189	1.475	<0.0001
Age (per year)	1.01	1.007	1.014	<0.0001
Sex (males vs. females)	1.30	1.190	1.422	<0.0001
Ethnicity (Jews vs. Arabs)	0.87	0.765	0.981	0.024
Smoking (ever vs. never)	1.12	1.015	1.240	0.024
Histology (adenocarcinoma)				
vs. squamous	1.14	1.003	1.304	0.045
vs. other	1.18	1.022	1.372	0.024

Table 4
Mutation specific HR of advanced NSCLC treated with TKIs diagnosed at Clalit's National Personalized Medicine Laboratory.

	HR	95%CI -	95%CI +	P
EGFR WT - with no TKIs treatment	1.00			
EGFR mutation ex. 19Del with TKIs ($n=220$)	0.50	0.420	0.585	<0.0001
EGFR mutation L858R with TKIs ($n=142$)	0.61	0.502	0.783	<0.0001
EGFR mutation G719X with TKIs ($n=17$)	0.70	0.410	1.176	0.175
EGFR mutation L861Q with TKIs ($n=16$)	0.63	0.354	1.103	0.105
EGFR mutation E709Q with TKIs ($n=7$)	0.87	0.388	1.928	0.723
EGFR mutation T790M* with TKIs ($n=2$)	0.70	0.174	2.794	0.611

* Refers to mutation status at baseline and not at progression on TKIs.

for ever smoking) and tumor histology (HR=1.14 for squamous cell), the overall survival advantage of TKI-treated cases with EGFR mutated tumors stayed statistically significant and clinically meaningful **(**HR=0.63, 0.55–0.71, $p<0.0001$) **(Table 3).**

A similar direction of survival advantage of EGFR-mutated lung cancer cases was seen for all reported EGFR mutations. Statistically significant survival benefit was demonstrated for the two common genetic alterations – exon 19 deletions (HR=0.50, 0.42–0.59, $p<0.0001$) and the L858R mutation in exon 21 (HR=0.61, 0.50–0.78, $p<0.0001$) (**Table 4**). Other types of EGFR mutations were relatively rare, with small sample sizes and statistical significance was not reached.

Discussion

This report provides real life data on the long term survival benefit of TKI treatment for advanced NSCLC patients with sensitizing EGFR mutations. Our data reiterates the significant and meaningful advantage provided by targeted agents for EGFR-mutated NSCLC, demonstrated in randomized controlled trials [3–5,10], or in other reports of small number of cases [12] or a series of cases with brain metastases in China [11]. It further provides insight into the significant differences in the effect of TKIs by age, ethnicity, gender, smoking and histology, identifying Jewish, non-smoking women with adenocarcinoma as the subgroup with the highest benefit.

Recent studies focusing on G-CSFR using global proteomics and phosphoproteomics experiments have found activation of unique kinases downstream of mutated G-CSFR and then showed the significance of TKI against leukemia [20–23]. Similarly, EGFR mutated lung cancer cells had been shown to harbor activated kinases downstream of EGFR [24,25].

The lack of effect, or even possibly an adverse effect, of TKIs on survival of NSCLC cases with wild-type EGFR is in apparent contrast to the previously reported benefit of erlotinib as 2nd or 3rd line treatment for advanced NSCLC in comparison to placebo, as seen in the BR.21

study [26] and further emphasizes the need to follow the rationale for this biologically tailored treatment. Although several reports demonstrate equivalence of EGFR TKIs to chemotherapy, more recent studies focusing on wild type EGFR patients demonstrate the superiority of chemotherapy over TKI [27]. Our report is the first to our knowledge to demonstrate the lack of efficacy of EGFR TKIs for wild type patients in real world data.

While the disease free survival benefit of treating advanced EGFR-mutated NSCLC with TKIs has repeatedly been proven, long-term follow-up studies of the relatively recently introduced TKI-treatments, including evaluation of overall survival have naturally been sparse [28–32] The Israeli health system in general and the Clalit HMO specifically are ideal for real world data analysis. The national health insurance covering the entire population, the wide population coverage of Clalit health services, and the utilization of the Israeli unique identification number to allow near complete follow up data provide high validity to our results. All reported testing was performed in a single central lab, also contributing to a high level of validity. Importantly, individual patient-level clinical data from the centralized and standardized EMR of Clalit were available regarding treatment, tumor histology, demography (race, gender, age) and smoking history. Our follow-up of up to 8 years provides a look into the experience of a full cohort, diagnosed and treated under similar conditions and with as near to complete as possible clinical and survival data.

Our data are in line with former reports of an increased proportion of EGFR mutations in lung tumors in females, in non- smokers and in adenocarcinomas [33–37]. This manuscript adds to current evidence [30,35] that treatment with TKIs in these sub-groups, with high mutation rates, actually led to a significantly better overall survival in a model which included all studied confounders. Former studies have shown disease free survival advantage, irrespective of EGFR status, for non-smokers and females [38–42]. We demonstrate that being a male smoker, as well as being treated for non-adenocarcinoma NSCLC is associated with lower survival under TKIs treatment, compared to non-smokers, whether females or males, with adenocarcinomas.

In addition, we found Israeli Arabs to have a worse outcome among the EGFR mutation carriers treated with TKIs. These data of suggested ethnicity-dependent outcome could have major implications for the health system.

Our results could be flawed if either the EGFR mutation analysis or the clinical data were wrong. The National molecular laboratory of Clalit has performed thousands of EGFR tests with an overall mutation rate of 17% which is in line with the expected mutation rate in a Caucasian population [33]. Furthermore, as formerly stated, we have found the expected histology, gender and smoking related differences in mutation rate. Since all treatments with TKIs had to be centrally approved by Clalit's gate-keeping personnel, and all treatments provided were entered into a central pharmacy database, it is unlikely that treatments were either missed or overstated. The demonstrated large survival benefit seen among TKIs users harboring EGFR mutations is in line with former experimental data and further supports the validity of both molecular and clinical data elements.

In our long-term full follow-up of an historical prospective cohort, treatment of patients with EGFR-mutated NSCLCs with TKIs led to a statistically significant and clinically meaningful survival benefit which lasted for at least five years and had a differential effect depending on the demographic, behavioral and clinical characteristics of the cases. The cohort described in this manuscript can serve as a benchmark for real-world data of EGFR mutation positive NSCLC patients and efficacy of EGFR TKI treatment.

Declaration of Competing Interest

None of the authors has any conflict of interests to report.

CRediT authorship contribution statement

Gad Rennert: Conceptualization, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Maya Gottfried:** Data curation, Investigation, Writing - review & editing. **Hedy S Rennert:** Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Flavio Lejbkowicz:** Formal analysis, Investigation, Writing - review & editing. **Meira Frank:** Formal analysis, Investigation, Writing - review & editing. **Ilana Cohen:** Formal analysis, Investigation, Writing - review & editing. **Shiri Kelt:** Formal analysis, Investigation, Writing - review & editing. **Abed Agbarya:** Data curation, Writing - review & editing. **Elizabeta Dudnik:** Data curation, Writing - review & editing. **Julia Dudnik:** Data curation, Writing - review & editing. **Rivka Katznelson:** Data curation, Writing - review & editing. **Moshe Mishali:** Data curation, Writing - review & editing. **Natalie Maimon Rabinovich:** Data curation, Writing - review & editing. **Hovav Nechushtan:** Data curation, Writing - review & editing. **Amir Onn:** Data curation, Writing - review & editing. **Shoshana Keren Rosenberg:** Data curation, Writing - review & editing. **Mariana Wollner:** Data curation, Writing - review & editing. **Alona Zer:** Data curation, Writing - review & editing. **Jair Bar:** Data curation, Writing - original draft, Writing - review & editing. **Naomi Gronich:** Conceptualization, Formal analysis, Methodology, Writing - original draft, Writing - review & editing.

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