14th Annual University of Pennsylvania Conference on Statistical Issues in Clinical Trials

Subgroup Analysis in Clinical Trials: Opportunities and Challenges

Finding the *(biomarker-defined)* subgroup of patients who benefit from a novel therapy: no time for a game of hide and seek

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DISCLOSURES

I have no financial relationships to disclose.

- and -

I will not discuss off label use and/or investigational use in my presentation.

- and -

The views expressed represent my own and do not necessarily represent the views or policies of the National Cancer Institute, National Institutes of Health, or the U.S. Department of Health and Human Services.

Having worked in oncology for the last 25+ years, the examples in my talk will all relate to cancer, but hopefully it will be clear how the statistical principles apply more generally.

Drug development in the era of biomarker-driven precision medicine

- Q1: Does the drug benefit any patients?
- Q2: If the drug does not benefit entire patient population, is there a subset that it does benefit?
- Q3: If the drug benefits only a subset, is there a biomarker (or "signature") that defines the subset?
  - "Predictive" (therapy selection / treatment effect modifier) biomarker
- Q4: If a biomarker is needed, how do we measure it?

Biomarker challenges

An optimal treatment-selection biomarker test might not be ready when a new therapeutic is ready for evaluation in a clinical trial

- Insufficient understanding of biology or mechanism of action of drug to confidently identify a biomarker or signature
- Not sure how to best measure the biomarker
- For quantitative biomarkers, unsure of best clinical cut-off
- Difficulties developing a robust, reproducible assay

Clinical trial design considerations when faced with uncertainty about a biomarker-defined subgroup most likely to benefit

- Extracting a candidate biomarker from the literature or preliminary data
- Biomarker assay reproducibility
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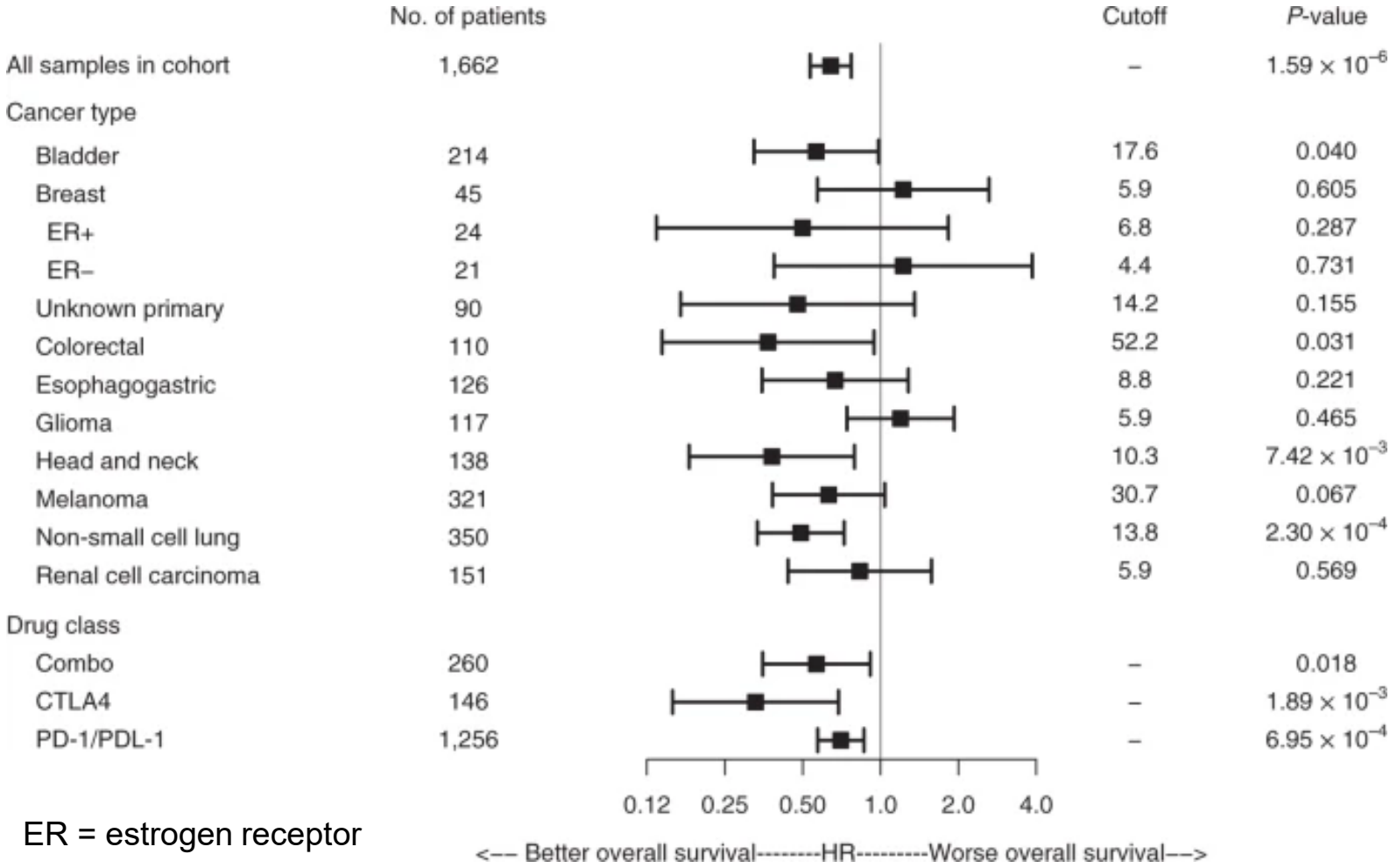
Extracting a candidate biomarker from the literature or preliminary data

- Preclinical studies – how to extrapolate biomarker results from preclinical models to humans, lack of preclinical models (e.g., for immunotherapies)
- Phase I/II clinical trials - small samples sizes, research grade biomarker assays, earlier endpoints (e.g., tumor response), heterogeneous patients (e.g., different tumor types), often non-randomized
- Retrospective biomarker evaluation on "convenience" specimens – generally heterogeneous patients & treatments, poor study design and analysis, unreliable/incomplete data, biomarker assays not sufficiently described
- Retrospective biomarker evaluation on specimens from similar completed trials – possibly different cancer types or disease stages, or different therapies in same class, different version of the biomarker assay

Tumor Mutational Burden (TMB) cut-offs, association with overall survival, by tumor type

Samstein et al. *Nat Genet* 2019;51:202-206 (Figure 2); MSKCC institutional case series



ER = estrogen receptor

- TMB = nonsynonymous mutational load/burden (mutations per megabase) by MSK-IMPACT assay using both tumor and germline DNA
- Cut-off defined as top 20%-tile of TMB for each tumor type, which varied widely
- Observed positive prognostic effect of TMB-High vs. TMB-Low on overall survival after ICI treatment for most tumor subtypes and all drug classes
- Two-sided log-rank P value for the comparison of TMB-High and TMB-L survival curves < 0.05 for 4/11 tumor types (influenced by sample size)

2 subgroup factors: tumor type and TMB (High vs. Low)

Tumor Mutational Burden (TMB) association with tumor response, by FoundationOne CDx

Marabelle et al. *Lancet Oncology* 2020;21:1353-1365

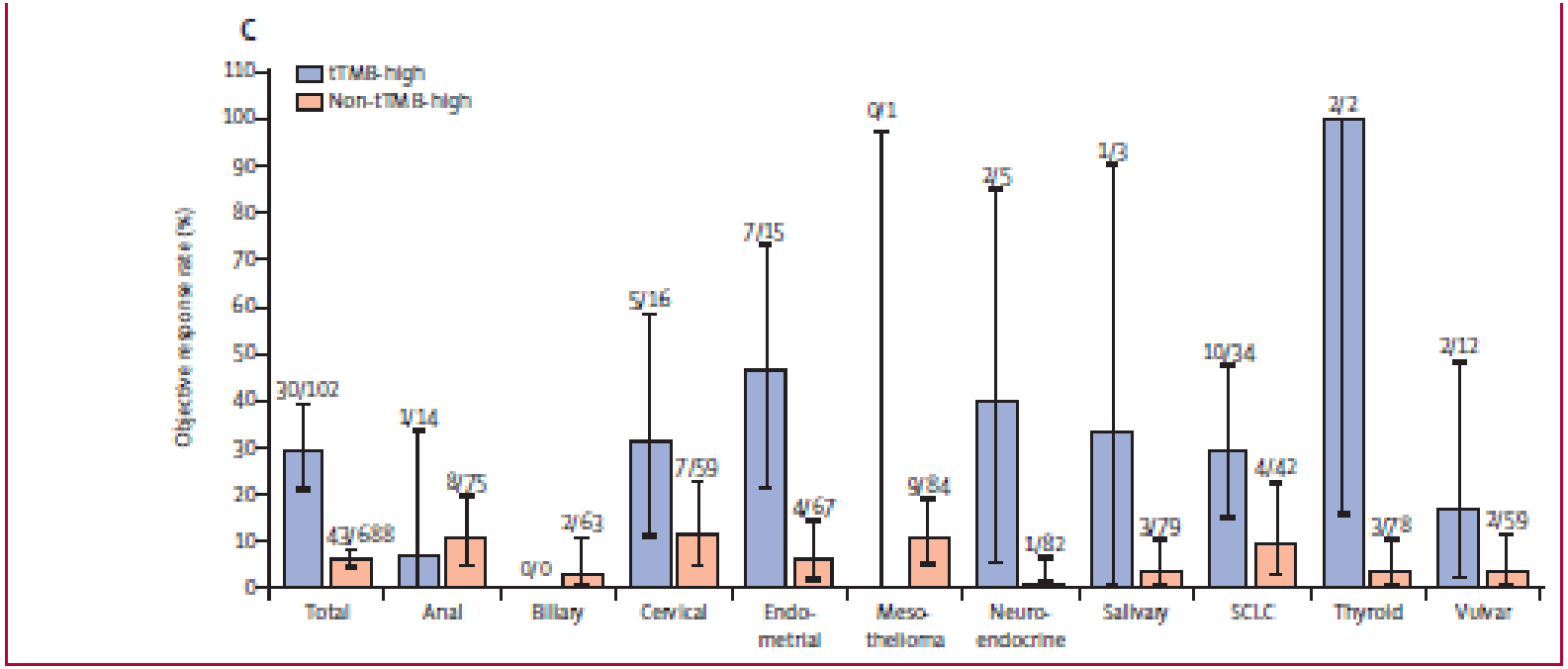


Figure 2: **Responses as per RECIST version 1.1, assessed by Independent central review, in the efficacy population**
(A) Best change from baseline in size of target lesions. Bars along the x-axis represent individual patient data, and dashed lines indicate changes in tumour size that are 20% above and 30% below baseline. 11 patients in the tTMB-high group and 69 patients in the non-tTMB-high group were excluded from this analysis because they did not have at least one evaluable post-baseline image assessment. (B) Kaplan-Meier estimates of duration of confirmed response. Vertical lines on curve denote censored patients. (C) Confirmed objective response rate by tumour type, with bars showing the proportion of patients with objective response and whiskers showing 95% CIs, with participant numbers indicated by n/N. MSI-H=high microsatellite instability. RECIST=Response Evaluation Criteria in Solid Tumors. SCLC=small-cell lung carcinoma. tTMB-high=high tissue tumour mutational burden. *Non-MSI-H category is comprised of patients with microsatellite instability-low or microsatellite stable tumours.

- TMB = DNA base substitution mutations (including synonymous) per megabase, by FoundationOne CDx assay
- Prespecified definition of TMB-High was TMB ≥ 10 across all tumor types
- Objective response rate (ORR, pooled across tumors types) is 6% (95% CI 5-8%) for TMB-Low vs. 29% (95% CI 21-39%) for TMB-High
- In TMB-H, ORRs by tumor type range from 0% (no TMB-High) to ≈ 47% (discounting 2/2 = 100% in thyroid)
- In TMB-L, ORRs by tumor type range from ≈ 3% to ≈ 12%
- **Supported tumor agnostic FDA approval for pembrolizumab with selection by FoundationOne CDx**

Multicohort, open-label, phase 2 KEYNOTE-158 study of pembrolizumab

Tumor Mutational Burden (TMB) association with tumor response, by MSK-IMPACT

Valero et al. *JAMA Oncology* 2021;7(5):739-743

Table. Distribution of Patients According to Tumor Mutational Burden by Cancer Type

Cancer type	Overall No. of patients	TMB-L (<10 mutations per Mb)		TMB-H (≥10 mutations per Mb)	
		Patients, No. (%)	Response rate, %	Patients, No. (%)	Response rate, %
NSCLC	663	459 (69)	21	204 (31)	35
Melanoma	214	101 (47)	32	113 (53)	58
Kidney	92	92 (100)	40	0	NA
Sarcoma	84	80 (95)	16	4 (5)	75
Bladder	82	56 (68)	9	26 (32)	35
Head and neck	74	61 (82)	21	13 (18)	31
Gastric	67	62 (93)	31	5 (7)	20
SCLC	54	32 (59)	16	22 (41)	27
Hepatobiliary	53	49 (92)	12	4 (8)	0
Colorectal	50	43 (86)	5	7 (14)	14
Endometrial	47	44 (94)	21	3 (6)	67
Esophageal	45	41 (91)	32	4 (9)	50
Pancreatic	36	34 (94)	3	2 (6)	0
Mesothelioma	35	34 (97)	18	1 (3)	0
Ovarian	29	28 (97)	11	1 (3)	100
Unknown primary	28	23 (82)	9	5 (18)	20
Breast	25	23 (92)	9	2 (8)	100
Total	1678	1262 (75)	21	416 (25)	41

- MSKCC institutional case series of 1678 patients included 16 tumor types treated with anti-PD-1 or anti-PD-L1 therapy
- TMB = nonsynonymous mutational burden by MSK-IMPACT assay (mutations per megabase)
- TMB-High was pre-specified as TMB ≥ 10 across all tumor types
- Response rates generally higher in TMB-High subgroup (11/16 tumor types, excluding unknown). However, high-TMB proportion and magnitude of association between TMB and response rates varied widely across tumor types.

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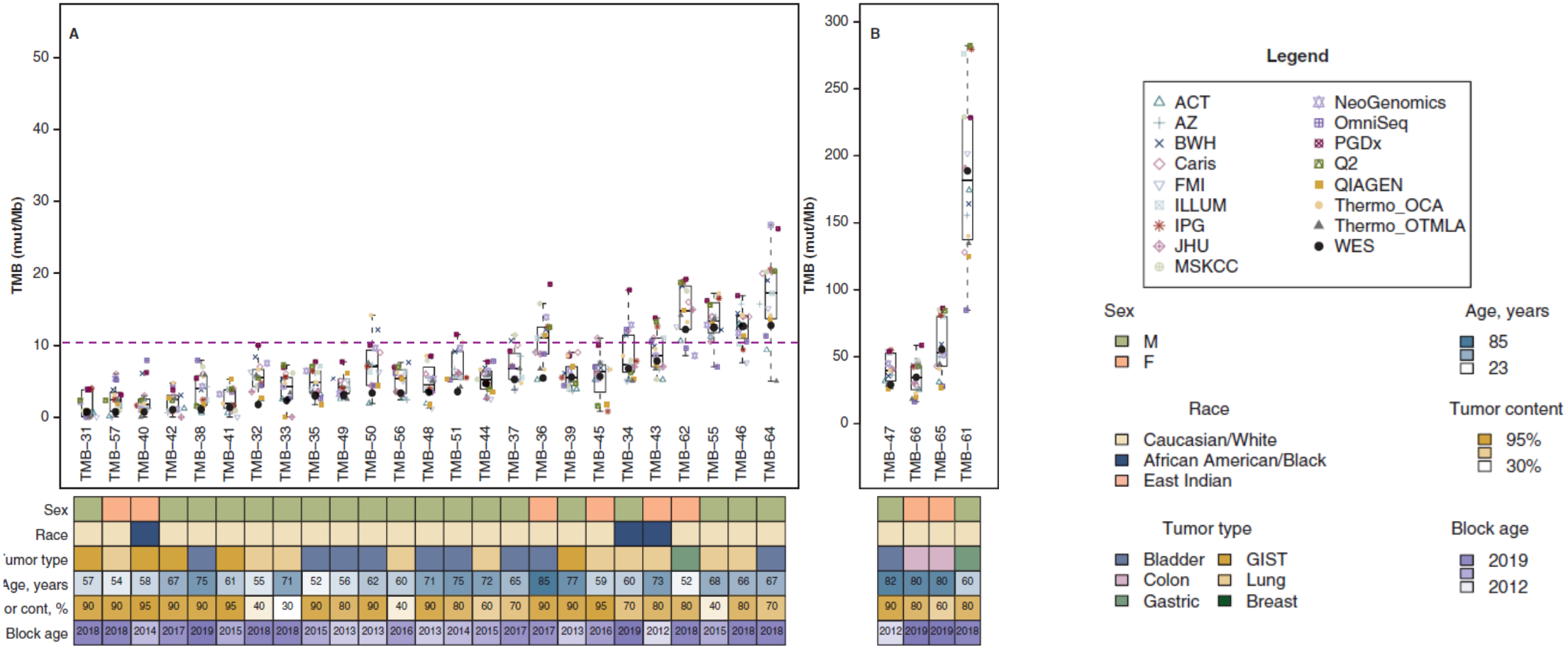
Different bioinformatic pipelines and algorithms produce variable results (and may change over time)

Example: Tumor Mutational Burden (TMB) by 17 different tests

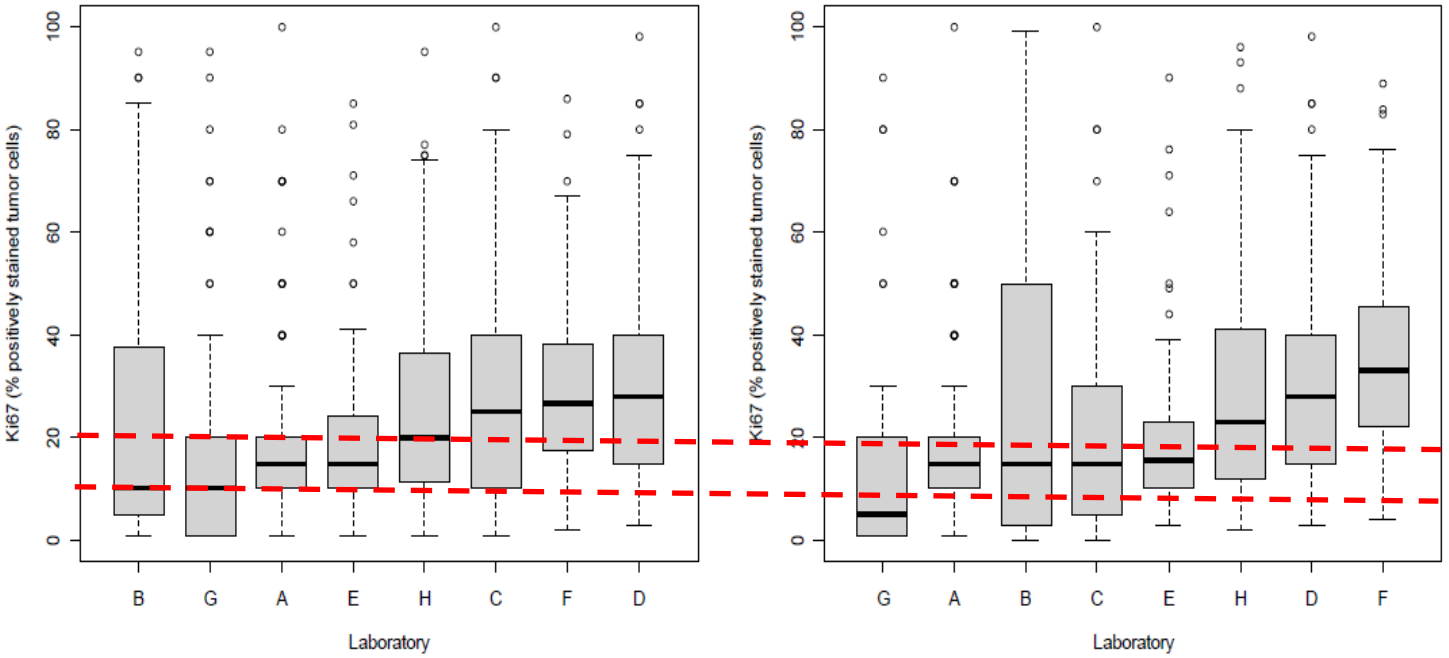
Variability due to different assay methods and bioinformatic pipelines



Vega et al. *Annals of Oncology* 2021;32(12):1626-1636 (Friends of Cancer Research TMB Harmonization Project)

Ki67 reproducibility across laboratories using different staining and scoring methods

Ki67 (% positive invasive tumor cells), 8 labs assessing different TMA sections, same set of 100 breast tumors



Centrally stained, locally scored
Median range: 10% to 28%
ICC: 0.71, 95% CI=(0.47,0.78)

Locally stained, locally scored
Median range: 5% to 33%
ICC: 0.59, 95% CI=(0.37,0.68)

Polley et al. *J Natl Cancer Inst* 2013;105:1897-1906

FDA approval summary for abemaciclib with endocrine therapy for high-risk early breast cancer

- Indication for abemaciclib + endocrine therapy **limited to** patients with ≥ 4 pathologic positive axillary lymph nodes (pALN) or 1-3 pALN and tumor histologic grade 3 and/or tumor size ≥ 50 mm whose tumors had **Ki-67 ≥ 20%**
- FDA simultaneously approved Ki-67 companion diagnostic (CDx) Ki67 MIB-1 IHC pharmDx (Dako Omnis, Carpinteria) that was used in trial.
- **Concerns that labs likely to use home brew Ki67 assays rather than the approved CDx!**

Royce et al. *J Clin Oncol* 2022; online

Clinical trial design considerations when faced with uncertainty about a biomarker-defined subgroup most likely to benefit

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Statistical design and analysis considerations for prospective biomarker-based subgroup testing

- To enrich or not enrich (with or without subsequent subgroup testing)
- Managing multiple subgroups
  - Pre-specified vs. post hoc
  - Type I error control
  - Nested vs. adjacent subgroup testing

Clinical trial enrichment

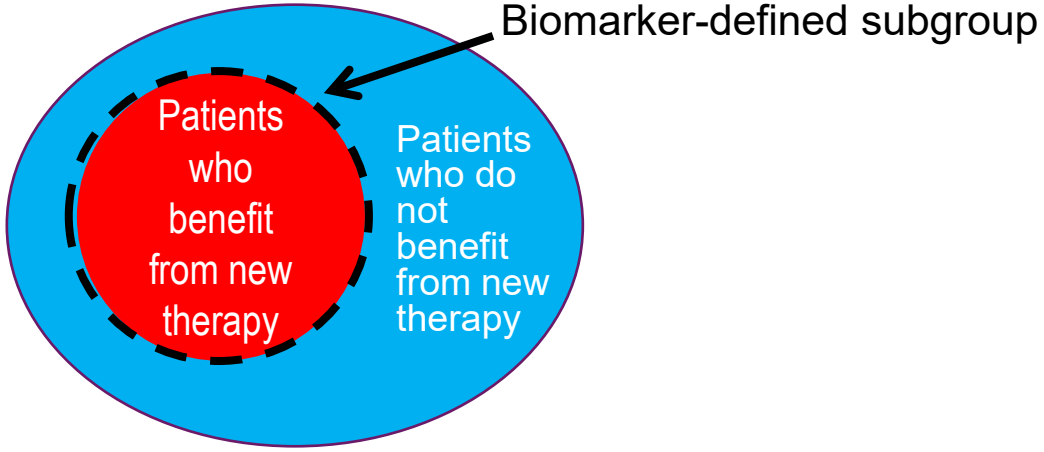
"Prospective use of any patient characteristic to select a study population in which detection of a drug effect (if one is in fact present) is more likely than it would be in an unselected population"

- Reduce inter-patient and intra-patient heterogeneity
- Prognostic enrichment strategies
  - More events ⇒ more statistical power
- **Predictive enrichment strategies – choosing patients more likely to respond to the drug treatment (e.g., use of treatment-selection biomarker)**

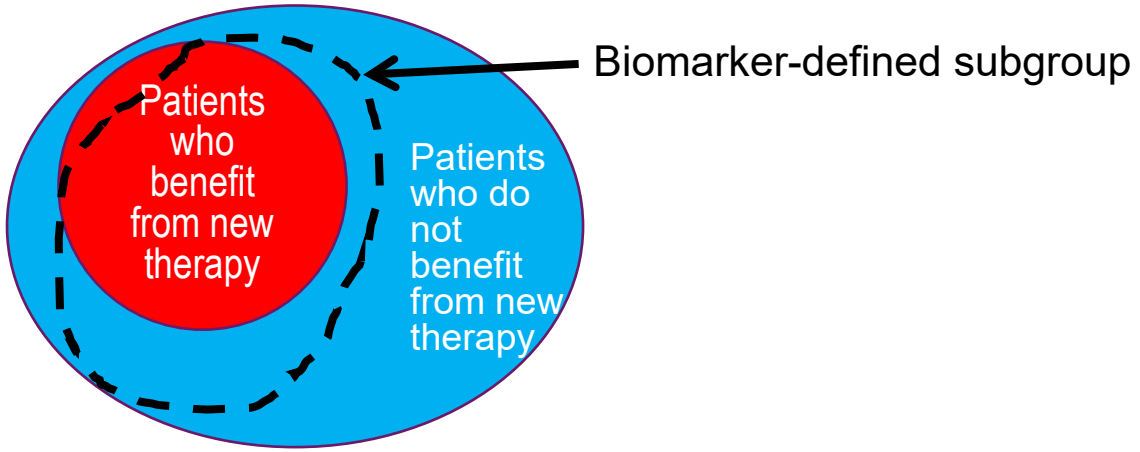
http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm332181.pdf

Considerations in use of a biomarker for up-front clinical trial enrichment

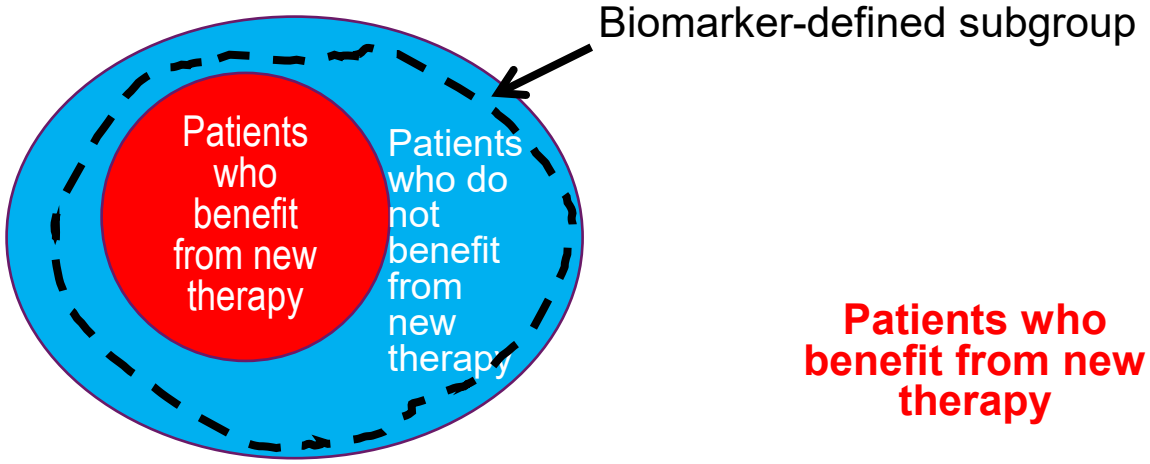
Precision medicine



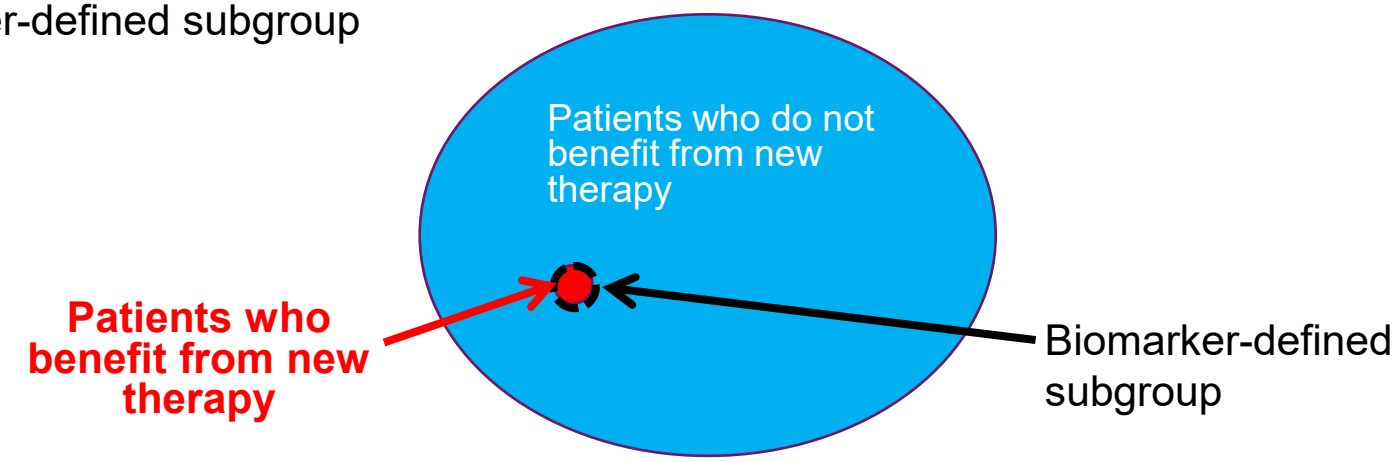
Biomarker very useful for enrichment



Biomarker minimally useful for enrichment



Trial extremely challenging even with a good enrichment biomarker



Principles for statistical management of multiple subgroups in a definitive clinical trial

- Most rigorous analysis pre-specifies subgroups (be judicious in choice and number) and controls overall type I error (e.g., partition α)
- Adaptations to subgroup testing or enrichment after trial initiation may be needed (e.g., due to external data), but ***must be made blinded*** to accruing outcome data

  https://www.fda.gov/regulatory-information/search-fda-guidance-documents/adaptive-design-clinical-trials-drugs-and-biologics-guidance-industry

- **Hierarchical testing of treatment effect in "biomarker-positive" subgroup followed by testing in overall (ITT) population is a generally *flawed approach***.

Rothmann et al. *Drug Information Journal* 2012;46(2):175-179
Kim & Prasad. *European Journal of Cancer* 2021;155:163e167
Freidlin & Korn. *J Natl Cancer Inst* 2022;114(2):187–190

Hierarchical testing example 1: Checkmate 649

Janjigian et al. *Lancet* 2021;398:27-40

- Randomized, open-label, phase 3 trial
- First-line, nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma
- Dual primary endpoints, two-sided significance levels (type I error) of 0·03 allocated to OS and 0·02 to PFS. Upon superiority of OS in patients with a PD-L1 CPS* ≥ 5, OS was ***hierarchically tested*** in patients with a PD-L1 CPS ≥ 1 with a fraction of α (50% α transmitted=0·015), followed by all randomly assigned patients (ITT, 100% α transmitted=0·015).

*CPS = combined positive score by Dako PD-L1 immunohistochemistry 28-8 pharmDx assay (Dako, Santa Clara, CA, USA)

Hierarchical testing example: Checkmate 649 Overall survival (OS)

Janjigian et al. *Lancet* 2021;398:27-40

	Nivo + chemo			Chemo alone			
Group	**n**	**12-mo OS (95% CI)**	**Med OS (mo) (95% CI)**	**n**	**12-mo OS (95% CI)**	**Med OS (mo) (95% CI)**	**HR CI p-value**
CPS ≥ 5	473	57% (53–62)	14.4 (13.1–16.2)	482	46% (42–51)	11·1 (10.0–12.1)	HR 0.71 98.4% CI (0.59–0.86) p<0.0001
CPS ≥ 1	641	56% (52–59)	14.0 (12.6–15.0)	655	47% (43–51)	11.3 (10.6–12.3)	HR 0.77 99.3% CI (0.64–0.92) p<0.0001
ITT	789	55% (51–58)	13.8 (12.6–14.6)	792	48% (44–51)	11.6 (10.9–12.5)	HR 0.80 99.3% CI (0.68–0.94) p=0.0002

HR

increasing

ASCO 2021 presentation (abstract #4002) showed HR=0.94 for CPS < 5

Hierarchical testing example: Checkmate 649 Progression free survival (PFS) Janjigian et al. *Lancet* 2021;398:27-40

	Nivo + chemo			Chemo alone			
Group	**n**	**12-mo PFS (95% CI)**	**Med PFS (mo) (95% CI)**	**n**	**12-mo PFS (95% CI)**	**Med PFS (mo) (95% CI)**	**HR CI p-value**
CPS ≥ 5	473	57% (53–62)	7.7 (7.0–9.2)	482	46% (42–51)	6.0 (5.6–6.9)	HR 0.68 98% CI (0.56–0.81) p<0·0001
CPS ≥ 1	641	56% (52–59)	7.5 (7.0–8.4)	655	47% (43–51)	6.9 (6.1–7.0)	HR 0.74 95% CI (0.65–0.85) (not tested)
ITT	789	55% (51–58)	7.7 (7.1–8.5)	792	48% (44–51)	6.9 (6.6–7.1)	HR 0.77 95% CI (0.68–0.87) (not tested)

HR ↓ increasing

ASCO 2021 presentation (abstract #4002) showed HR=0.93 for CPS < 5

Hierarchical testing example 2: MonarchE Trial

Randomized, multi-center, open-label, **two-cohort phase 3 trial** comparing **abemaciclib (CDK4/6 inhibitor) plus endocrine therapy (ET) to ET alone** in patients with hormone-receptor-positive (HR+) human epidermal growth factor receptor-negative (HER2-) early breast cancer (EBC) **at high risk of disease recurrence** on the basis of clinical or pathologic features or Ki-67 score

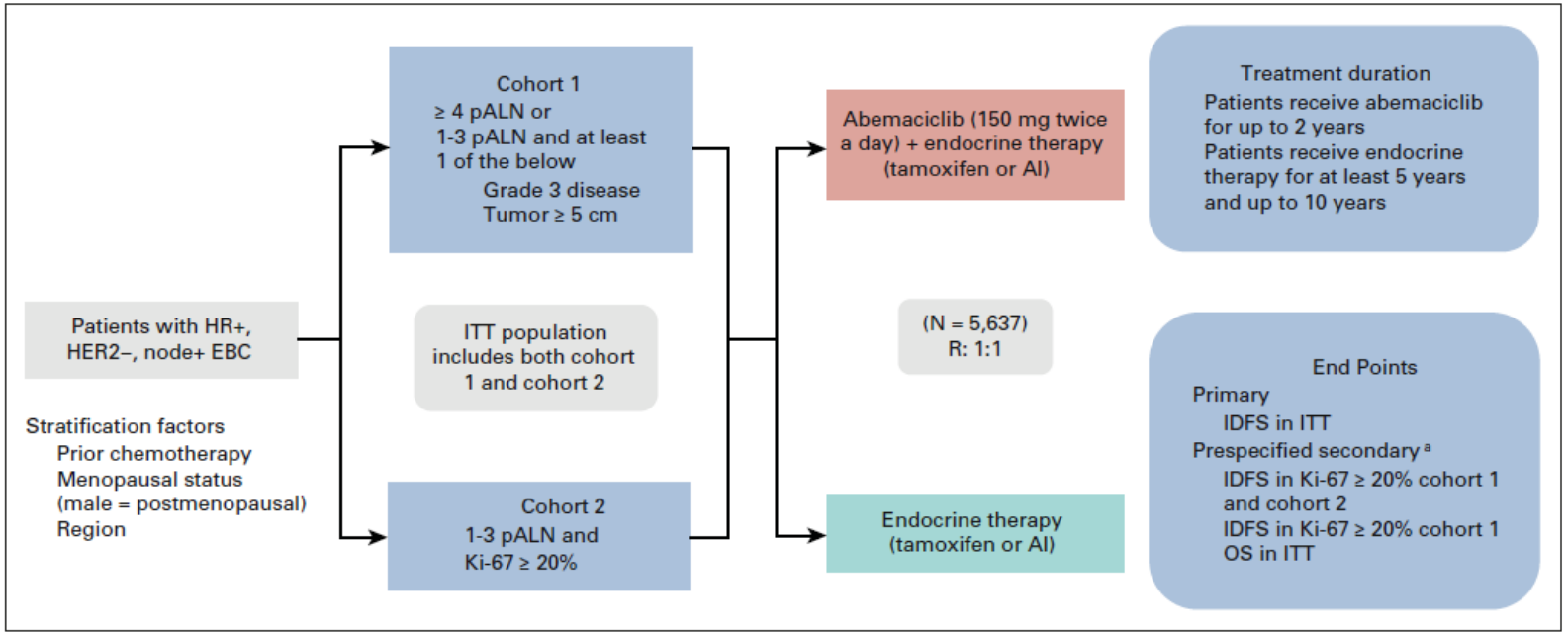


FIG 1. Trial schema of monarchE. Study design of monarchE, including key eligibility criteria and stratification factors, two enrollment cohorts, treatments arms (A = experimental—ET with abemaciclib *v* B = control—ET alone), treatment duration, and study end points. [a]A gated hierarchical testing strategy to control for type 1 error included three additional end points, IDFS in patients with Ki-67 score ≥ 20% from cohorts 1 and 2, followed by IDFS in patients with a Ki-67 score ≥ 20% from cohort 1 alone, and finally OS in the ITT population. AI, aromatase inhibitor; EBC, early breast cancer; ET, endocrine therapy; HER2–, human epidermal growth factor receptor 2–negative; HR+, hormone receptor–positive; IDFS, invasive disease-free survival; ITT, intent-to-treat; OS, overall survival; pALN, pathologic positive axillary lymph nodes; R, random assignment.

- **Cohort 1:** patients with ≥ 4 pathologic positive axillary lymph nodes (pALN) or 1-3 pALN and tumor histologic grade 3 and/or tumor size ≥ 50 mm
- **Cohort 2:** patients with 1-3 pALN and **Ki-67 score ≥ 20%**

Royce et al. *J Clin Oncol* 2022; published online Jan 27, 2022: DOI https://doi.org/10.1200/JCO.21.02742

Johnstone et al. *J Clin Oncol* 2020;38:3987-3998

Harbeck et al. *Annals of Oncology* 2021;32(12):1571-1581

Hierarchical testing example 2: MonarchE Trial

Statistical plan

- Study powered to test the intent-to-treat (ITT) population (Cohort 1 + Cohort 2) for IDFS.
- Gated hierarchical testing strategy included three additional end points:
  - IDFS in patients with Ki-67 score ≥ 20% from cohorts 1 and 2
  - IDFS in patients with Ki-67 score ≥ 20% from cohort 1 alone
  - OS in the ITT population
- Two interim analyses and one final efficacy analysis for IDFS were planned, as well as two interim analyses and one final OS analysis
- Two subgroup factors: cohort 1 vs. 2 and Ki67 (≥ 20% vs. < 20%)

TABLE 1. Timing of Prespecified IDFS and OS Analyses in monarchE to Date

Prespecified Analysis	Target/Actual No. of Events	Timing	End Points Meeting Statistical Significance
Interim IDFS 1 (IA1)	195/203	September 27, 2019	
Interim IDFS 2 (IA2)	293/323	March 16, 2020	IDFS in ITT ($p < 0.026$)
Final IDFS	390/395	July 8, 2020	IDFS in Ki-67 ≥ 20% (C1 and C2) IDFS in Ki-67 ≥ 20% (C1)
OS Interim 1 (OS IA1)[a]	—/186	April 1, 2021	

Abbreviations: C1, cohort 1; C2, cohort 2; IA, interim analysis; IDFS, invasive disease-free survival; ITT, intent-to-treat; OS, overall survival.
[a]Additional OS analyses will be conducted.

Hierarchical testing example 2: MonarchE Trial

Prespecified analysis	Endpoints meeting statistical significance
Interim IDFS 1 (IA1)	
Interim IDFS 2 (IA2)	IDFS in ITT
Final IDFS	IDFS in Ki67 ≥ 20% (Cohorts 1+2) IDFS in Ki67 ≥ 20% (Cohort 1)
OS Interim 1 (OS IA1)	

FDA approved use of abemaciclib + ET in patients at high risk of recurrence meeting the monarchE cohort 1 criteria and whose tumors are Ki-67 ≥ 20% on the basis of the simultaneously approved CDx (Ki67 MIB-1 IHC pharmDx (Dako Omnis, Carpinteria).

Royce et al. *J Clin Oncol* 2022; online Jan 27, 2022

- Despite IDFS in ITT statistically significant favoring abemaciclib + ET at all analyses, **ITT OS analysis at all time points (IA2 , final IDFS, and OS IA1 data cut-offs) favored ET only arm.**
- In both final IDFS analysis and OS IA1, **remaining subgroups in hierarchy (Ki-67 ≥ 20%)**, each had statistically significant IDFS results deepening with time and **OS HRs that numerically favored the abemaciclib plus ET arm** with HR < 1.
- However, cohort 2's enrollment began approximately 11 months after cohort 1, and cohort 2 alone represented only 9% of total patients (with few events); **statistically significant IDFS improvement in Ki-67 ≥ 20% population (cohorts 1 and 2 combined) was driven by cohort 1.**

Recommended alternatives to hierarchical approach

Rothmann et al. *Drug Information Journal* 2012;46(2):175-179

Freidlin & Korn. *J Natl Cancer Inst* 2022;114(2):187–190

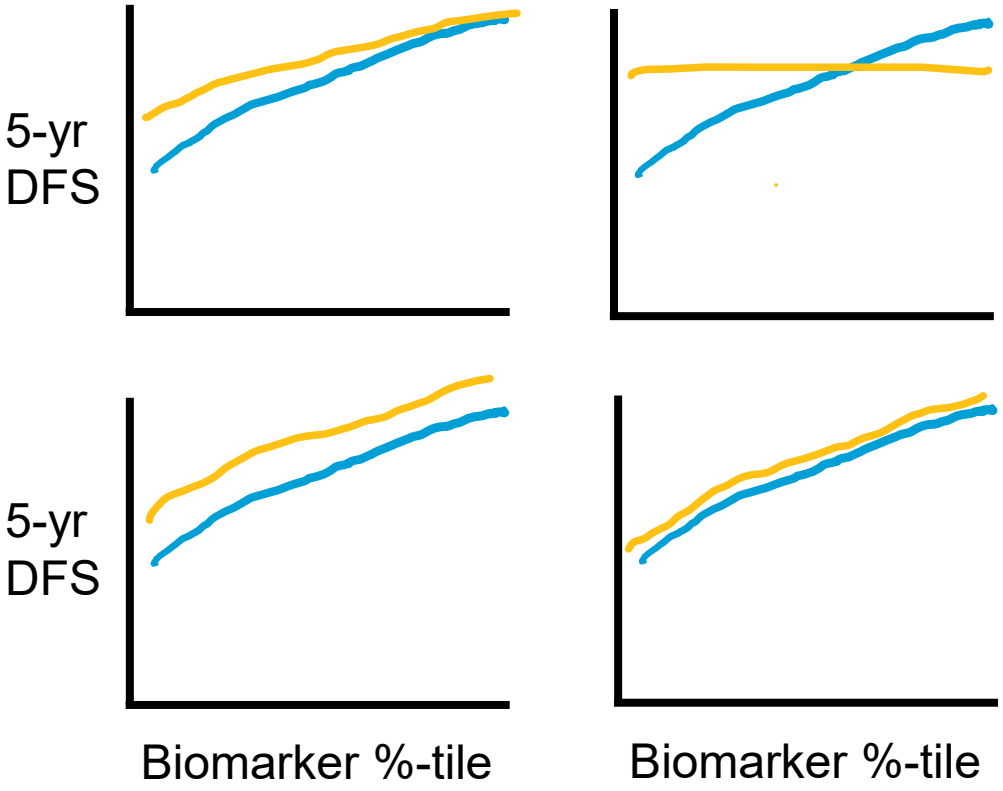
- **For conclusions of favorable efficacy in biomarker-positive subgroup to be extrapolated to include the biomarker-negative subjects**
  - Address the multiplicity caused by subgroup testing
  - Sufficient data to obtain reliable treatment effect estimate in biomarker negative subgroup
  - Estimated treatment effect in biomarker negative subgroup should be clinically relevant and at least as large as needed to achieve "statistical significance" in an ITT analysis
- **When no expectation of large difference in treatment effects across subgroups, primary analysis should be limited to the entire ITT population**
  - Subgroups can be investigated in an exploratory manner
- **When genuine uncertainty (equipoise) about whether biomarker associated with magnitude of treatment effect, biomarker stratified design with early futility analysis for biomarker negative subgroup may be warranted**

Recommended graphical displays

When subgroups defined by cut-off(s) on a continuous biomarker, examine treatment effect on the biomarker continuum

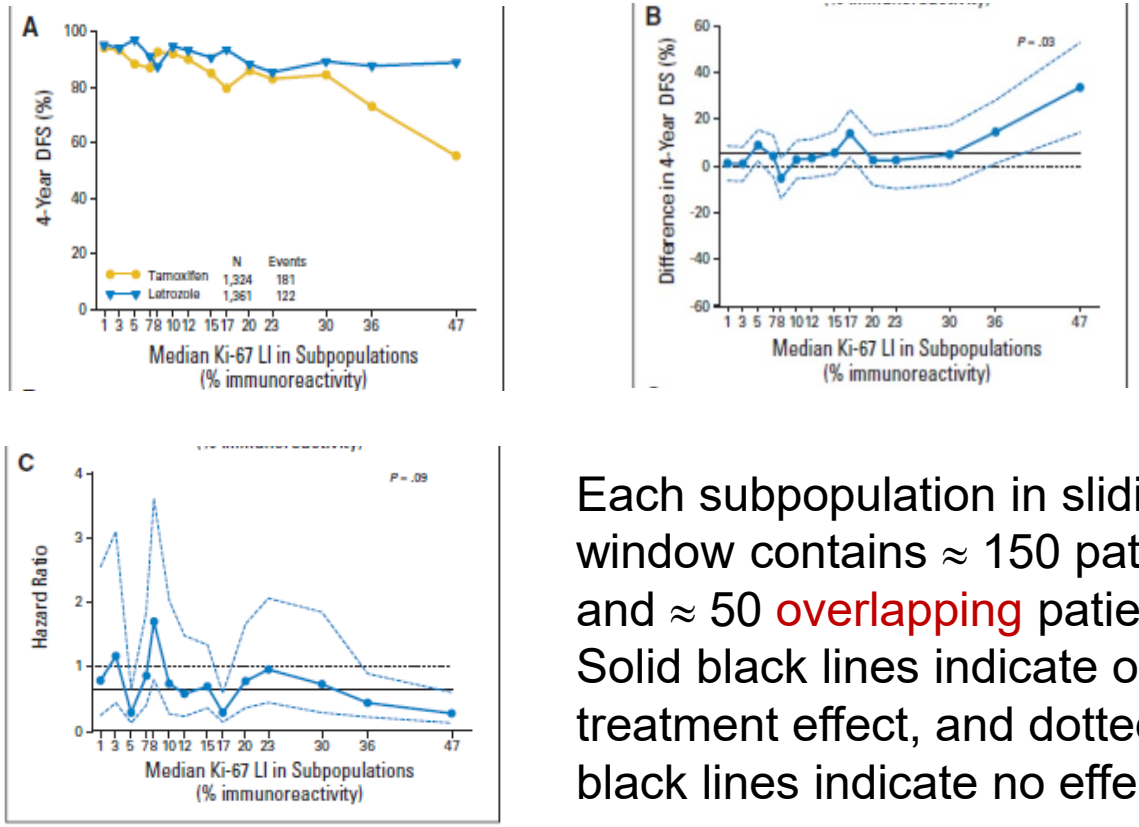
Predictiveness curves

Janes et al. *Ann Intern Med* 2011;154:253-259



Subgroup Treatment Effect Pattern Plot (STEPP)

Lazar et al. *J Clin Oncol* 2010; 28: 4539-4544



Each subpopulation in sliding window contains ≈ 150 patients and ≈ 50 overlapping patients. Solid black lines indicate overall treatment effect, and dotted black lines indicate no effect.

Summary remarks

- **Precision medicine brings statistical challenges of increasing number and decreasing size of subgroups; hopefully small subgroup sample sizes are counterbalanced by considerably larger magnitudes of treatment effect.**
- **Conclusions about treatment effects in subgroups should be based on careful consideration of multiple factors or evidence sources**
  - Requires understanding various biomarkers, assays, therapeutic agents, and clinical populations
  - Delicate balance between consideration of multiple factors and pitfalls of *post hoc* analyses
    - Amatya AK et al. *Clinical Cancer Research 2021;*27(21):5753-5756
- **Need complete and transparent reporting of any studies involving biomarkers**
  - Present treatment effects in disjoint/adjacent biomarker-based subgroups rather than nested
  - For continuous biomarkers, present treatment effects along the biomarker continuum
  - Completely describe biomarkers or algorithms for identifying subgroups to allow assessment of comparability across studies and reproducibility in practice
    - REMARK reporting guidelines: Sauerbrei et al. *J Natl Cancer Inst* 2018;110(8):djy088

THANK YOU