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RODRIGO GONÇALVES

DESENVOLVIMENTO DE UM MÉTODO PADRONIZADO PARA AVALIAÇÃO DO BIOMARCADOR KI-67 EM PACIENTES COM CÂNCER DE MAMA, SUA VALIDAÇÃO CLÍNICA E AVALIAÇÃO DO IMPACTO ECONÔMICO DE SUA ADOÇÃO NO SUS

DEVELOPMENT AND CLINICAL VALIDATION OF A STANDARDIZED METHOD TO EVALUATE KI-67 IN BREAST CANCER PATIENTS AND EVALUATION OF THE ECONOMIC IMPACT OF ITS ADOPTION IN BRAZIL'S PUBLIC HEALTH SYSTEM (SUS)

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ORIENTADOR: LUÍS OTÁVIO ZANATTA SARIAN

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RESUMO

Introdução: O câncer de mama é uma doença heterogênea, dividida em subtipos moleculares, com tratamentos e prognósticos distintos. Na indisponibilidade de testes moleculares, a identificação desses subtipos pode ser feita baseada na imunoistoquímica dos receptores de estrogênio (RE) e progesterona (RP), HER-2 e Ki-67. O Ki-67 é utilizado para diferenciar entre subtipo Luminal A e B, e também como um instrumento de avaliação de resposta à tratamento endócrino neoadjuvante. Seu uso, entretanto, sofre duras críticas devido à falta da padronização de sua metodologia.

Objetivos: Desenvolver uma padronização para a avaliação do Ki-67 em pacientes com câncer de mama e avaliar o impacto econômico de seu uso como ferramenta de escolha de diferentes tratamentos.

Metodologia: Desenvolvemos um método de avaliação de Ki-67 assistido por computador focado em reproduzir os pontos de corte (PC) 2.7%, necessário para o cálculo do índice prognóstico de tratamento endócrino pré-operatório (PEPI), e 10%, necessário para identificação precoce de não-respondedores. A métrica primária avaliada foi a concordância de desfechos clínicos entre dois patologistas. Tal método de avaliação foi empregado na análise do estudo ACOSOG Z1031A que recrutou pacientes com câncer de mama RE+, HER-2 negativo, localmente avançado para tratamento endócrino neoadjuvante. Utilizamos o modelo de Cox para avaliar se a sobrevida livre de doença foi diferente em pacientes com PEPI=0 (T1 ou T2, N0, Ki67 > 2.7%, RE Allred > 2) versus PEPI > 0. Finalmente desenvolvemos um modelo matemático para estimar os desfechos econômicos de diferentes estratégias de tratamento das pacientes com câncer de mama, RE+, HER2-, localmente avançado, baseadas na avaliação do Ki-67.

Resultados: O método de avaliação do Ki-67 foi empregado em casos T1/2 N0 dos estudos POL e P024. A concordância percentual positiva para o PC 2,7% foi 87.5% (IC95% 61.7- 98.5%); concordância percentual negativa 88.9% (IC95%: 65.3-98.6%). A avaliação de Ki-67 dos dois patologistas gerou curvas de sobrevida livre

de doença semelhantes (Log rank P=0.044 e P=0.055). Os dados para o PC 10% no estudo POL foram concordância percentual positiva 100%; concordância percentual negativa 93.55% (IC95%: 78.58-99.21%). As curvas de sobrevida foram concordantes (Log rank P=0.0001 e P=0.01). No estudo ACOSOG Z1031, nosso método foi capaz de identificar um grupo de pacientes com extremo baixo risco de recorrência após 5.5 anos de seguimento (HR [PEPI = 0 vs PEPI > 0], 0.27; IC95% 0.092 a 0.764). Nosso modelo mostra que, considerando as premissas adotadas, podem ser poupados R$32009,36 por paciente se utilizarmos a estratégia de tratamento endócrino neoadjuvante ao invés da estratégia padrão de tratamento.

Conclusão: O método de avaliação do biomarcador Ki-67 desenvolvido é eficiente e reprodutível. O score PEPI, baseado no valor de Ki-67 utilizando a metodologia desenvolvida, é capaz de identificar um grupo de mínimo risco de recidiva que pode ser manejado sem o uso de quimioterapia. O uso do Ki-67 no SUS como método de individualização de tratamento em pacientes com câncer de mama RE+, HER-2 negativo, localmente avançado pode resultar em economia importante de recursos.

Palavras-chave: 1. Neoplasias da mama; 2. Biomarcadores; 3. Custos e análise de custo

ABSTRACT

Introduction: Breast cancer is a heterogeneous disease divided in molecular subtypes, each with different treatment options and different outcomes. In the absence of gene expression tests, immunohistochemistry of estrogen receptor (ER), progesterone receptor (PR), HER-2 and Ki-67 should be used to determine molecular subtypes. Ki-67 should be used to differentiate Luminal A from Luminal B, and also as a predictive biomarker of neoadjuvant endocrine treatment response. Its use is still criticized due to lack of methodology standardization.

Objetives: To develop a standard evaluation method to the biomarker Ki-67 in breast cancer patients and assess the economical impact of tailored treatment based on Ki-67 evaluation.

Methodology: Computer-assisted Ki-67 assay assessment focused on reproducing a 2.7% Ki-67 cut-point (CP) required for calculating the Preoperative Endocrine Prognostic Index (PEPI). A CP of 10% for poor responder identification within the first month of neoadjuvant endocrine treatment was also evaluated. Clinical outcome concordance for two independent Ki-67 scores was the primary performance metric. The computer-assisted method was run in the ACOSOG Z1031A trial that enrolled postmenopausal women with locally advanced ER-positive (Allred score, 6 to 8), HER-2 negative, breast cancer for neoadjuvant endocrine treatment. Stratified Cox modeling was used to assess whether time to recurrence differed by PEPI = 0 score (T1 or T2, N0, Ki67 > 2.7%, ER Allred > 2) versus PEPI > 0 disease. Finally we developed a mathematical model to estimate the economic outcomes of different treatment strategies, in women with ER+, HER2 negative, stage 2 or 3 breast cancer based in Ki-67 evaluation.

Results: The final Ki-67 scoring approach was run on T1/2 N0 cases from the P024 and POL trials. The percent positive agreement for the 2.7% CP was 87.5% (95% CI 61.7- 98.5%); percent negative agreement 88.9% (95% CI: 65.3-98.6%). Minor discordance did not affect the ability to predict similar relapse-free outcomes (Log Rank P=0.044 and P=0.055). The data for the 10% early triage CP in the POL trial were percentage positive agreement 100%; percent negative agreement 93.55%

(95% CI: 78.58-99.21%). The independent survival predictions were concordant (Log rank P=0.0001 and P=0.01). In the ACOSOG Z1031 trial, after 5.5 years of median follow-up, our method was able to identify a group of very low risk of relapse (recurrence hazard ratio [PEPI = 0 v PEPI > 0], 0.27; 95% CI, 0.092 to 0.764). Our model shows that, given the stated assumptions, the incremental cost savings were R$32009.36 per patient for the neoadjuvant endocrine treatment strategy compared to the standard-of-care strategy.

Conclusion: Our computer-assisted Ki-67 assay is efficient and reproducible. Using the proposed Ki-67 methodology, PEPI score can identify patients with very low risk of relapse that can be managed without chemotherapy. The implementation of this tailored treatment for locally advanced ER+ HER2- breast cancer based on Ki-67 evaluation can lead to important resources savings.

Keywords: 1. Neoplasms; 2. Biomarkers; 3. Cost and cost analysis

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LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

ASCO	Sociedade Americana de Oncologia Clínica
AZ	Arizona
CCM	Cirurgia conservadora de mama
CDIS	Carcinoma ductal *in situ*
EUA	Estados Unidos da América
FDA	*Food and Drug Administration*
HER-2	Receptor do fator de crescimento epidérmico 2
IHQ	Imunoistoquímica
Ki-67	Marcador celular de proliferação Ki-67
PEPI	*Preoperative Endocrine Prognostic Index*
RE	Receptor de estrogênio
RE+	Receptor de estrogênio positivo
RH	Receptor hormonal
RH+	Receptor-hormonal positivo
RNA	Ácido ribonucleico
RP	Receptor de progesterona
SUS	Sistema Único de Saúde
T-DM1	Trastuzumabe-emtansine
TRCM	Tempo para recorrência de câncer de mama

LISTA DE SÍMBOLOS

%	Porcentagem
-	Negativo
+	Positivo
<	Menor
≤	Menor igual
>	Maior
≥	Maior igual
®	Registrada

SUMÁRIO

I. INTRODUÇÃO

O câncer de mama é uma doença heterogênea, dividida em subtipos moleculares, com diferentes opções de terapia adjuvante e com prognósticos distintos. Essa divisão em subtipos moleculares foi proposta por Perou et al em 2000[1] após a análise de expressão gênica de 8102 genes em 65 espécimes cirúrgicos de 42 pacientes com câncer de mama. Nesse estudo os autores determinaram a existência de 5 subtipos moleculares a saber: luminal A; luminal B; basalóide; com amplificação de HER-2 e; normal. Em estudos subsequentes[2], foi demonstrado que o subtipo normal na realidade tratava-se de uma análise equivocada de amostras com baixo conteúdo tumoral e elevado conteúdo de tecido mamário normal adjacente[3].

Quando analisada a sobrevida das pacientes de acordo com os subtipos moleculares, foi demonstrado que pacientes com tumores do subtipo Luminal A apresentavam melhor prognóstico em 5 anos, ao passo que pacientes com tumores do subtipo basalóide apresentavam o pior prognóstico dentre as classes apresentadas[4]. Mais do que uma classificação prognóstica, a classificação em subtipos moleculares também é preditiva de resposta a diferentes tratamentos possíveis[5].

Pacientes com tumores do subtipo Luminal A terão pouco benefício com o uso de quimioterapia adjuvante e terão maior benefício do uso de terapia endócrina adjuvante[6]; pacientes com tumores do subtipo Luminal B apresentarão um benefício claro do uso de quimioterapia adjuvante seguido de terapia endócrina adjuvante por um período mínimo de 5 anos[7]; pacientes com tumores do subtipo com amplificação de HER-2 apresentarão benefício no uso de quimioterapia associada a trastuzumabe[8-14] e, mais recentemente, a outras drogas como o lapatinib[15], pertuzumabe[16] e trastuzumabe-emtansine (T-DM1)[17, 18]; e pacientes com tumores do subtipo basalóide carecem de terapias-alvo específicas recebendo quimioterapia padrão baseada em doxorrubicina, ciclofosfamida e taxanes[7].

A identificação dos subtipos moleculares através da determinação da expressão gênica, conforme descrito nos estudos pioneiros sobre o tema, não é prática rotineira na clínica. Existem dois testes moleculares disponíveis comercialmente capazes de identificar os subtipos moleculares com base em expressão gênica, o Prosigna®

(NanoString Technologies, Seattle, WA 98109, EUA)[2] e o BluePrint® (Agendia Inc., Irvine, CA 92618, EUA)[19]. Tratam- se de ensaios diagnósticos *in vitro* que utilizam dados de expressão gênica, ponderados em conjunto com variáveis clínicas, a fim de gerar categorias de risco e avaliar a chance de recorrência à distância em 10 anos. Os testes são aplicáveis em mulheres na pós-menopausa com linfonodos axilares negativos (Estádios I ou II) e receptor-hormonal positivo (RH +). Esses testes medem a expressão de RNA extraído de amostras tumorais fixadas em formol e embutidas em parafina (FFPE). Contudo, seus custos tornam-se obstáculos quase intransponíveis em boa parte do mundo, inclusive no Brasil, onde a maior parte das pacientes depende do Sistema Único de Saúde (SUS)[20]. Dessa forma, a comunidade científica tem realizado esforços significativos para a adoção de biomarcadores substitutos na determinação dos subtipos moleculares do câncer de mama.

Na reunião de St. Gallen realizada em 2015, ficou estabelecido que na indisponibilidade de testes moleculares para definição de subtipos moleculares, seria aceita a classificação baseada na análise imunoistoquímica (IHQ) do receptor de estrogênio (RE), receptor de progesterona (RP), HER-2 e Ki-67 (tabela 1)[21]. Desses biomarcadores, os 3 primeiros são bem aceitos e utilizados rotineiramente na prática clínica após extensos estudos que determinaram sua utilidade e estabeleceram sua padronização, culminando na incorporação nos manuais de conduta da Sociedade Americana de Oncologia Clínica (ASCO) [22, 23]. O uso do Ki-67, entretanto, ainda sofre duras críticas pela comunidade científica devido à falta da padronização de sua metodologia tanto pré-analítica como analítica.

O Ki-67 foi descrito pela primeira vez em 1983 por Gerdes et al[24]. O antígeno Ki-67 é expresso durante todas as fases do ciclo celular, exceto em G0, e seus níveis atingem o máximo durante a mitose. Desde então, a expressão de Ki-67 foi utilizada no manejo de diferentes neoplasias, entre elas o câncer de ovário, colo uterino e o câncer de mama. No manejo do câncer de mama, como foi descrito anteriormente, o Ki-67 é usado principalmente na diferenciação entre os subtipos moleculares Luminal A e Luminal B. No consenso de especialistas realizado em St. Gallen em 2015, a maior parte dos participantes votou que o ponto de corte para essa diferenciação deveria ser entre 20-29%[21].

Mais do que um biomarcador que apenas diferencia entre câncer Luminal A e B, o uso do Ki-67 parece ter maior valor quando usado como um instrumento de avaliação de resposta à endocrinoterapia, principalmente no cenário do tratamento neoadjuvante. Em pacientes com câncer de mama estádios II ou III, com tumores do subtipo luminal, a estratégia desenvolvida por Ellis et al consistia em iniciar o tratamento com endocrinoterapia e, após um período de 16 a 18 semanas, as pacientes eram submetidas à cirurgia e os parâmetros de tamanho tumoral (T), status linfonodal (N), receptor de estrogênio (RE) e Ki-67 eram novamente avaliados[25, 26]. Após análise multivariada confirmando a associação entre esses marcadores e a sobrevida das pacientes, foi criado o score PEPI (Preoperative Endocrine Prognostic Index) (tabela 2) que se mostrou uma excelente ferramenta baseada no Ki-67 para determinação de prognóstico. O score PEPI foi então validado na população de pacientes do estudo IMPACT[27]. Nesse estudo, mulheres portadoras de câncer de mama estádio II ou III, RE+ e HER-2 negativo foram randomizadas para receberem tamoxifeno, anastrozol ou uma combinação das duas drogas como tratamento neoadjuvante. Essas pacientes foram submetidas a biópsias seriadas, sendo uma ao diagnóstico, uma após 2 a 4 semanas de tratamento neoadjuvante e a última na análise da peça cirúrgica. Através da análise dessas amostras, ficou demonstrado o valor prognóstico do PEPI score nesse grupo de pacientes. Mais do que isso, com a análise das biópsias de 2 a 4 semanas após o início do tratamento neoadjuvante, levantou-se a hipótese que pacientes apresentando um valor de Ki-67 superior a 10%, dificilmente alcançariam um score PEPI com valor zero e, portanto, teriam indicação de quimioterapia adjuvante (tabela 3). Convém ressaltar, que além do score PEPI, fortemente baseado no Ki-67, existem testes de expressão gênica que foram desenvolvidos exatamente para identificar pacientes de bom prognóstico que possam ser tratadas sem a necessidade de quimioterapia adjuvante. Os principais testes disponíveis para essa finalidade são o Oncotype Dx ®[28], o MammaPrint ®[29] e o Prosigna ® [2]. Apesar de atualmente estarem sendo testados em pacientes com câncer de mama RE+, Her-2 negativo, localmente avançado, seu uso nesse cenário clínico ainda não é embasado por evidências científicas sólidas.

Em uma revisão de literatura publicada em um periódico do grupo Nature em 2012, Goncalves et al identificaram que a análise do biomarcador Ki-67 e sua utilização no score PEPI em estudos de tratamento endócrino neoadjuvante era capaz de predizer o resultado de estudos de tratamento endócrino adjuvante[30]. Dessa forma, estudos

adjuvantes dispendiosos, com necessidade de tamanho amostral na casa dos milhares de pacientes e com a necessidade de anos de seguimento deveriam ser ativados apenas após a realização de estudos de tratamento neoadjuvante que são capazes de gerar resultados de maneira mais ágil com o uso de biomarcadores substitutos, como o score PEPI. Tal hipótese foi mais uma vez confirmada com a recente publicação dos resultados do estudo FACE [31], no qual o letrozol apresentou mesma eficácia que o anastrozol no tratamento adjuvante, resultado antecipado pelo estudo de tratamento neoadjuvante ACOSOG Z1031[32] que descreveremos a seguir.

O ACOSOG Z1031[32] foi um estudo de fase II no qual 377 mulheres com câncer de mama estádios II ou III, RE+, HER-2 negativo foram randomizadas para receber exemestano, anastrozol ou letrozol neoadjuvantes e o objetivo primário foi a taxa de resposta clínica. Variações nos níveis de Ki-67 em vigência de tratamento neoadjuvante e o score PEPI foram objetivos secundários desse estudo. Do ponto de vista de resposta clínica, foi demonstrado com base nas variações de Ki-67 e no score PEPI que letrozole e anastrozole são biologicamente equivalentes. A validação do PEPI score nessa casuística comprovou seu valor, com uma clara separação entre as curvas de sobrevida no grupo que alcançou score PEPI igual a zero versus o grupo que não atingiu score PEPI igual a zero[33]. Numa coorte adicional desse estudo, a avaliação do Ki-67 serviria para triar pacientes para diferentes modalidades de tratamento. Se o valor de Ki-67 fosse menor ou igual a 10%, a paciente continuaria recebendo endocrinoterapia neoadjuvante por 16-18 semanas; se o valor de Ki-67 fosse maior que 10%, a paciente receberia quimioterapia neoadjuvante ou cirurgia imediata (figura 1). Os resultados desse estudo, que é parte integrante e fruto dessa tese, foram recentemente publicados no Journal of Clinical Oncology[34].

O estudo ALTERNATE (NCT01953588) segue um desenho semelhante à coorte Z1031B e tem por objetivo primário a validação prospectiva do PEPI score com o seguimento das pacientes em vigência de endocrinoterapia adjuvante, último passo para sua introdução na prática clínica[35]. Estima-se um tamanho amostral de 2820 pacientes e o recrutamento está em andamento.

Como foi demonstrado até aqui, existe um claro papel para o uso do Ki-67 no manejo das pacientes com câncer de mama subtipo molecular luminal. Entretanto, a comunidade científica ainda não está completamente convencida de que esse

biomarcador é reprodutível em diferentes locais do mundo[36]. Protocolos de manejo de pacientes, como o NCCN[7], ainda não recomendam o uso do Ki-67 enquanto esse biomarcador não passar por uma maior validação analítica e padronização na sua leitura. Em 2011, Dowsett et al [37] publicaram uma primeira tentativa de padronização da metodologia da avaliação do Ki-67, incluindo variáveis analíticas, pré-analíticas e método de interpretação e graduação. Entretanto, os métodos descritos naquela publicação são muito trabalhosos e demandam muito tempo para avaliação individual de cada caso. Esse mesmo grupo, em 2013, propôs um estudo para a avaliação de reprodutibilidade do biomarcador no qual 8 laboratórios de grupos renomados na comunidade científica receberam 100 amostras de câncer de mama[36]. Cada laboratório foi responsável pela realização das reações de IHQ e pela interpretação das lâminas. Os resultados foram pouco satisfatórios com uma reprodutibilidade entre laboratórios apenas moderada (correlação intra-classe=0.59, IC95%= 0.37 a 0.68). Em um novo esforço em 2015, o mesmo grupo propôs um novo estudo com o objetivo de aumentar a concordância na avaliação do Ki-67[38]. Dessa vez o grupo estabeleceu instruções claras para a realização das reações de IHQ e também para o método de avaliação das lâminas. Apesar disso, algumas discrepâncias persistiram inclusive nos pontos de corte com relevância clínica. A recomendação do grupo foi de que mais esforços para padronização seriam necessários antes da adoção desse biomarcador na prática clínica.

Como não existe um método padrão para a avaliação do biomarcador Ki-67, esse estudo pretende desenvolver uma padronização para sua avaliação desde a fase pré-analítica até sua graduação e avaliar o impacto econômico do uso desse marcador como ferramenta de escolha de diferentes tratamentos.

Tabela 1. Definição dos Subtipos Moleculares segundo avaliação imunoistoquímica

Subtipo Molecular	Receptor de Estrógeno	Receptor de Progesterona	HER-2	Ki-67
Luminal A	+	+/-	-	≤ 14%
Luminal B	+	+/-	+/-	>14%
Her-2	-	-	+	N/A
Basal	-	-	-	N/A

Legenda: + = positivo; - = negativo; a combinação das características imunoistoquímicas pode definir os subtipos moleculares do câncer de mama.

Tabela 2. Índice prognóstico pós terapia endócrina neoadjuvante (PEPI score)

Biomarcador	Sobrevida livre de doença		Sobrevida específica de câncer	
	HR	Pontos	HR	Pontos
Tamanho do tumor (pT)				
T1/2	-	0	-	0
T3/4	2,8	3	4,4	3
Status Linfonodal				
Negativo	-	0	-	0
Positivo	3,2	3	3,9	3
Ki-67				
0%-2,7%	-	0	-	0
>2,7%-7,3%	1,3	1	1,4	1
>7,3%-19,7%	1,7	1	2,0	2
>19,7%-53,1%	2,2	2	2,7	3
>53,1%	2,9	3	3,8	3
Receptor de estrogênio (Score Allred)				
0-2	2,8	3	7,0	3
3-8	-	0	-	0

Legenda: a análise multivariada das características tumorais, após endocrinoterapia neoadjuvante, demonstrou razões de risco (HR) maiores para tumores maiores que 5cm, status linfonodal positivo, receptor de estrógeno Allred 0-2 e Ki-67>2.7%. A soma dos pontos atribuído a cada característica determina o score PEPI.

Tabela 3. Associação entre valor de Ki-67 após 2 semanas e obtenção de score PEPI igual a zero no estudo IMPACT [39]

Ki-67 após 2 semanas	N (%)	Ki-67 na peça cirúrgica	PEPI Mediana (IQR)	N PEPI Score 0 (%)	N Eventos Sobrevida Livre de Recidiva (%)
> 10%	32 (24%)	15.9% (7.5 - 27.5)	4 (2.0-4.2)	0/32 (0%)	9/35 (26%)
≤ 10%	101 (76%)	2.4% (0.8 - 6.2)	3 (1.0-4.0)	21/101 (21%)	13/118 (11%)
Valor de p (teste)		P=0.001 (Wilcoxon)	P=0.001 (Wilcoxon)	P=0.004 (Fisher's) test)	P=0.008 (Log Rank)

Legenda: Pacientes que apresentaram Ki-67>10% após 2 semanas de endocrinoterapia neoadjuvante não obtiveram score PEPI=0 e apresentaram maior risco de recidiva no estudo IMPACT[39].

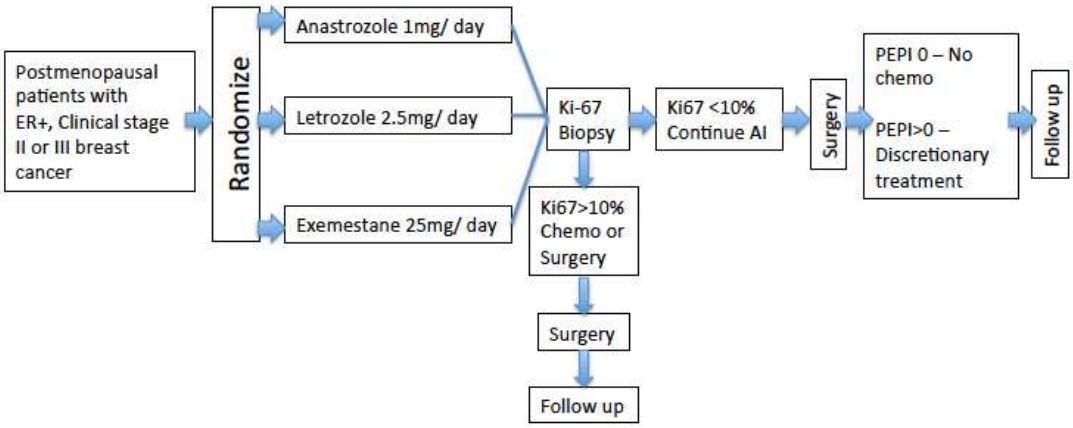

Figura 1. Desenho do estudo Z1031 coorte B [40]

II. OBJETIVOS

2.1. Objetivo Geral

Desenvolver um método padronizado para a avaliação através de imunoistoquímica do biomarcador Ki-67 em pacientes com câncer de mama do subtipo molecular Luminal e avaliar o impacto econômico da sua possível adoção no Sistema Único de Saúde (SUS) como ferramenta para escolha do tratamento adjuvante.

2.2. Objetivos Específicos

- **Artigo1:** Desenvolver e validar um método analítico assistido por computador para o uso do Ki-67 em pacientes com câncer de mama subtipo luminal, avaliando sua correlação com sobrevida global das pacientes.
- **Artigo 2:** Validar o método analítico assistido por computador desenvolvido no artigo 1 em uma coorte de pacientes (ACOSOG Z1031 coorte B) na qual o biomarcador Ki-67 seria determinante para a individualização do tratamento, avaliando o seu impacto na sobrevida global e sobrevida livre de doença.
- **Artigo 3:** Avaliar a custo-efetividade do uso do biomarcador Ki-67 no Sistema Único de Saúde, utilizando como modelo o estudo ACOSOG Z1031 coorte B, no qual pacientes com câncer de mama subtipo luminal receberiam quimioterapia adjuvante baseado nos resultados do Ki-67 e do score PEPI, como alternativa ao tratamento padrão atual.

III. MATERIAIS E MÉTODOS

Esta tese consiste de três estudos interligados. O primeiro trata do desenvolvimento de um método padronizado para avaliação de Ki-67 em pacientes com câncer de mama. O segundo estudo trata da validação do método desenvolvido no primeiro artigo e sua aplicação em uma coorte de pacientes com câncer de mama onde a avaliação da resposta é dependente do resultado do Ki-67. O ultimo estudo é uma análise do impacto econômico no Sistema Único de Saúde combinando a aplicação da metodologia do primeiro estudo e a estratégia de tratamento desenvolvido no segundo estudo.

Os protocolos de pesquisa dos estudos POL[25], P024[27] e ACOSOG Z1031 [32] foram aprovados pelos comitês de ética das instituições responsáveis e todos os pacientes assinaram termos de consentimento livre e esclarecido para participação nos estudos.

Uma síntese das características metodológicas de cada estudo é apresentada a seguir, enquanto os detalhes podem ser avaliados nas publicações referentes a cada um.

3.1. Desenvolvimento de um método padronizado para avaliação do biomarcador Ki-67

Nesse estudo foi desenvolvido um método para avaliação de Ki-67 baseado na digitalização das lâminas de IHQ e análise delas por um software desenvolvido pela Ventana/Roche e aprimorado pelo nosso grupo. Utilizamos amostras de 61 pacientes com câncer de mama com linfonodos axilares comprometidos do estudo P024[27] para o treinamento do uso do scanner para digitalização de imagens e para o desenvolvimento do algoritmo de avaliação de Ki-67 baseado no software de análise de imagens. Para validação do ponto de corte de Ki-67 de 10%, utilizado para triagem de pacientes para quimioterapia, utilizamos biópsias de fragmento de agulha grossa de 66 pacientes submetidas a 4 semanas

de terapia endócrina neoadjuvante [41]. Para validação do ponto de corte do Ki-67 de 2,7%, necessário para o score PEPI, espécimes cirúrgicos de 58 pacientes com câncer de mama estádios patológicos 1 ou 2A dos estudos POL[25] e P024[27] foram utilizados.

Para a realização desse estudo, secções de 5 mícron de amostras dos estudos POL[25] e P024[27] foram coradas pelo método de hematoxilina e eosina e através de reações de IHQ para Ki-67 utilizando o anticorpo primário monoclonal de coelho CONFIRM anti-Ki-67 (30-9) como um reagente pré-diluído e padronizado para a plataforma Benchmark XT, de acordo com as instruções do fabricante (Ventana, Tucson, AZ). Cortes de amigdala foram utilizados como controles.

Para avaliação de Ki-67 utilizando o software de análise de imagens, lâminas foram digitalizadas utilizando o scanner iScan Coreo (Ventana). As imagens digitalizadas foram então submetidas à análise pelo software no qual o patologista selecionava áreas de interesse (AOI) sob um aumento de 4X utilizando as seguintes recomendações: 1) identificar a maior AOI representativa de tumor invasivo; 2) excluir carcinoma ductal *in situ* (CDIS), vasos e linfócitos; 3) evitar AOI em áreas necróticas ou peri-necróticas; 4) delimitar um mínimo de 3 e um máximo de 10 AOI. A análise das imagens foi feita utilizando o algoritmo Companion Algorithm Ki-67 (30-9), autorizado pelo FDA e o software Virtuoso (Ventana).

Para a contagem visual (VPC), fotografias de 3 campos selecionados aleatoriamente foram tiradas com um aumento de 40X e impressas em cores em papel com um gradeado de 0,635cmx0,635cm e foram avaliadas por 2 observadores. Cada avaliador faz a contagem do total de células e do número de células positivas para Ki-67 que eram cortadas pelas linhas do gradeado. Esse processo era repetido a cada terceira linha. Se um número mínimo de 200 células tumorais não fosse atingido na soma das 3 fotografias, então todas as células da lâmina seriam contadas. Entretanto, para nossa avaliação, um número mínimo de 200 células tumorais foi estabelecido como necessário.

Dois patologistas avaliaram independentemente as imagens digitalizadas de Ki-67 e selecionaram AOI para uso do software ou para VPC. A análise das

fotografias para VPC e os resultados de Ki-67 derivado das mesmas foram realizadas por técnicos treinados (um biólogo e um médico mastologista).

Análise de variância utilizando análise de scatter-plot foi calculada utilizando os coeficientes de correlação de Pearson e Spearman. A concordância entre dois patologistas para os pontos de corte de 2,7% e 10% foi avaliada utilizando análise de tabela de contingência 4x4 e coeficiente simples Kappa. O efeito prognóstico do score PEPI com resultado zero versus score PEPI com resultado diferente de zero foi determinado utilizando o método de Kaplan–Meier. O teste log-rank foi utilizado para determinação de significância estatística. Análises similares foram conduzidas para correlacionar o desfecho de sobrevida e os valores de Ki-67 após 4 semanas de tratamento (>10% vs $\leq$ 10%) no estudo POL[25].

3.2. Validação da metodologia padronizada para avaliação do biomarcador Ki-67 na coorte B do estudo ACOSOG Z1031

Pacientes recrutadas para o estudo ACOSOG Z1031B[34] foram submetidas a biópsia de agulha grossa após 2 a 4 semanas de tratamento endócrino neoadjuvante. Se o valor de Ki-67 fosse menor que 10% então a paciente seguiria em tratamento neoadjuvante por outras 12 a 14 semanas, seguida de cirurgia. Mulheres cujos valores de Ki-67 fossem maiores que 10% seriam triadas para quimioterapia neoadjuvante com um esquema recomendado pelo NCCN[7] ou iriam diretamente para cirurgia, de acordo com a avaliação do médico assistente. Se, em qualquer momento, existisse suspeita de progressão da doença, ultrassonografia e mamografia seriam realizados, e a endocrinoterapia neoadjuvante seria suspensa caso a progressão fosse confirmada. Para pacientes que obtiveram score PEPI igual a zero após o tratamento endócrino neoadjuvante, o manejo sem quimioterapia era recomendado, mas não obrigatório, de maneira a determinar a aceitabilidade dessa recomendação.

O endpoint primário do ACOSOG Z1031B[34] foi a taxa de resposta patológica completa (pCR) entre as mulheres que após duas semanas de tratamento endócrino neoadjuvante obtiveram valores de Ki-67 maiores que 10% e foram então

triadas para quimioterapia neoadjuvante. O endpoint secundário seria a correlação entre o score PEPI e a sobrevida. Tempo para recorrência de câncer de mama (TRCM) foi definida como o tempo entre a cirurgia e a primeira recorrência, local ou à distância. O TRCM foi estimado utilizando o método de Kaplan-Meier. O modelo Stratified Cox foi utilizado para avaliar se o TRCM foi diferente de acordo com o status do score PEPI (zero versus não-zero). O fechamento dos dados foi realizado em 11 de janeiro de 2016.

Um único patologista experiente (Donald Craig Allred) realizou as avaliações de Ki-67 ao longo do estudo ACOSOG Z1031B[34]. A análise retrospectiva do ACOSOG Z1031A[32] utilizou o scanner iScan Coreo (Ventana) com o software Companion Algorithm Ki-67 (30-9). A avaliação das imagens foi realizada de acordo com a metodologia desenvolvida em nosso primeiro artigo, sendo necessárias 3 a 10 AOI com um aumento de 4X, excluindo CDIS, vasos e linfócitos, e evitando áreas necróticas e peri-necróticas. A análise pelo software era então revista por um patologista (Sousan Sanati) para garantir que o software estava adequadamente diferenciando células benignas e malignas; caso contrário, aquele caso seria avaliado por VPC. A metodologia para VPC foi descrita acima.

3.3. Avaliação do impacto econômico da determinação da resposta ao tratamento endócrino neoadjuvante baseada no biomarcador Ki-67 no cenário do SUS

Nós desenvolvemos um modelo matemático, incluindo uma cadeia de Markov, para estimar os desfechos econômicos de uma estratégia de triagem de pacientes para quimioterapia baseado na resposta a terapia endócrina neoadjuvante, em mulheres com câncer de mama estádios 2 ou 3, RE+, HER2 negativo. Dados dos estudos ACOSOG Z1031 [42], NSABP-B18 [43] e do software Adjuvant!Online[44] foram utilizados para estimar a sobrevida dos pacientes em nosso modelo. O Adjuvant! Online é uma ferramenta baseada na internet desenvolvida para estimar a sobrevida em 10 anos de pacientes com câncer de mama, de acordo com diferentes opções de tratamento. Fontes extraídas da literatura nacional e internacional foram utilizadas para identificar a qualidade de vida

após diferentes eventos e tratamentos, e o custo das intervenções foi identificado no banco de dados do Ministério da Saúde brasileiro. Nós construímos um modelo de análise de decisões (utilizando o software TreeAge Pro) para avaliar duas alternativas de tratamento: na primeira os pacientes começariam o tratamento com endocrinoterapia neoadjuvante com um inibidor de aromatase; na segunda o paciente seguiria o tratamento padrão, sendo submetido a cirurgia inicialmente. Ambas modalidades de tratamento incluiriam cirurgia na mama e terapia endócrina adjuvante por 5 anos. Cirurgia conservadora de mama (CCM) ou mastectomia eram as modalidades de tratamento cirúrgico aceitas, de acordo com o tamanho tumoral no momento da cirurgia. Cada uma das alternativas de intervenção terminava em uma cadeia de Markov, no qual as pacientes navegariam por 4 estados de saúde: morte, sem evidência de doença, recidiva local ou metástase à distância. O modelo continuaria rodando até que todos as pacientes estivessem mortas por quaisquer causas. A estrutura do modelo de Markov é descrita em detalhes no corpo do artigo. Os dados de sobrevida utilizados para a cadeia de Markov foram extraídos do software Adjuvant!Online. Nós avaliamos os custos ao sistema de saúde pela perspectiva do provedor dos serviços, o Sistema Único de Saúde (SUS).

Além da análise de custo-efetividade, também realizamos o processo “one-way sensitivity analysis” para avaliar o impacto da falha do teste de Ki-67 nos custos do modelo.

IV. RESULTADOS

Artigo 1. Development of a Ki-67-based clinical trial assay for neoadjuvant endocrine therapy response monitoring in breast cancer

Artigo 2. Ki67 proliferation index as a tool for chemotherapy decisions during and after neoadjuvant aromatase inhibitor treatment for breast cancer: Results from the ACOSOG Z1031 Trial (Alliance).

Artigo 3. Cost-effectiveness analysis of locally advanced estrogen receptor-positive, HER-2 negative breast cancer care using a tailored treatment approach.

Artigo 1

----- Forwarded Message -----
From: Breast Cancer Research and Treatment (BREA)
<em@editorialmanager.com>
To: Rodrigo Goncalves <rodgon82@yahoo.com>
Sent: Thursday, May 25, 2017 12:22 PM
Subject: BREA-D-17-00642 - Submission Notification to co-author

Re: "Development of a Ki-67-based clinical trial assay for neoadjuvant endocrine therapy response monitoring in breast cancer"

Full author list: Rodrigo Goncalves; Katherine DeSchryver; Cynthia Ma; Yu Tao; Jeremy Hoog; Maggie Cheang; Erika Crouch; Neha Dahiya; Sousan Sanati; Michael Barnes; Luis Otávio Zanatta Sarian; John Olson; Donald Craig Allred; Matthew James Ellis

Dear Mr. Goncalves,

We have received the submission entitled: "Development of a Ki-67-based clinical trial assay for neoadjuvant endocrine therapy response monitoring in breast cancer" for possible publication in Breast Cancer Research and Treatment, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. Dr. Matthew James Ellis who will be able to track the status of the paper through his/her login.

If you have any objections, please contact the editorial office as soon as possible. If we do not hear back from you, we will assume you agree with your co-authorship.

Thank you very much.

With kind regards,

Springer Journals Editorial Office

Breast Cancer Research and Treatment

Development of a Ki-67-based clinical trial assay for neoadjuvant endocrine therapy response monitoring in breast cancer

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Abstract

Background: The recent publication of the ACOSOG Z1031 trial results demonstrated that Ki-67 proliferation marker-based neoadjuvant endocrine therapy response monitoring could be used for tailoring the use of adjuvant chemotherapy in ER+ HER2 negative breast cancer patients. In this paper we describe the development of the Ki-67 clinical trial assay used for this study.

Methods: Ki-67 assay assessment focused on reproducing a 2.7% Ki-67 cut point (CP) required for calculating the Preoperative Endocrine Prognostic Index (PEPI). A CP of 10% for poor endocrine therapy response identification within the first month of neoadjuvant endocrine treatment was also evaluated. Image analysis, to replace labor-intensive visual point counting (VPC), was assessed to increase the efficiency of the scoring process. Clinical outcome concordance for two independent Ki-67 scores was the primary performance metric.

Results: Discordant scores led to a triage approach where cases with complex histological features that software algorithms could not resolve were flagged for visual point counting (17%). The final Ki-67 scoring approach was run on T1/2 N0 cases from the P024 and POL trials (N=58). The percent positive agreement for the 2.7% CP was 87.5% (95% CI 61.7- 98.5%); percent negative agreement 88.9% (95% CI: 65.3-98.6%). Minor discordance did not affect the ability to predict similar relapse-free outcomes (Log Rank P=0.044 and P=0.055). The data for the 10% early triage CP in the POL trial was similar (N=66), the percentage positive agreement was 100%; percent negative agreement 93.55% (95% CI: 78.58-99.21%). The independent survival predictions were concordant (Log rank P=0.0001 and P=0.01).

Conclusions. We have developed an efficient and reproducible Ki-67 scoring system that was approved by the Clinical Trials Evaluation Program (CTEP) for NCI-supported neoadjuvant endocrine therapy trials. Using the methodology described here, investigators are able to identify a subgroup of patients with ER+ HER2 negative breast cancer that can be safely managed without the need of adjuvant chemotherapy.

Introduction

Biomarkers of cell proliferation are used to assess prognosis and response to cancer treatment and most clinical assays are based on Ki-67 immunohistochemistry (IHC) (1). The Ki-67 nuclear protein is present in proliferating cells but absent in cells in G0 (2). For breast cancer, Ki-67 analysis is relevant for estrogen receptor positive (ER+) early stage breast cancer (3-5) which presents as a spectrum of tumors with clinically indolent (Luminal A) or more aggressive features (Luminal B) (6). While the "luminal" classification is based on gene expression analysis, a Ki-67 cut-point of 14% of cells staining positive has been proposed as a surrogate for the distinction between luminal A and luminal B (7). This cut-point was considered clinically useful by the St. Gallen breast cancer consensus panel (8) but the concerns of the American Society of Clinical Oncology Tumor Marker Guideline Committee regarding the lack of rigor in Ki-67 scoring algorithms and the questionable validity of decision-making cut points has slowed clinical implementation (9).

Ki-67 analysis also has potential for monitoring endocrine therapy response, which requires testing a tumor specimen after endocrine treatment has been initiated, for example, in surgical specimens after neoadjuvant aromatase inhibition (10). The independent prognostic value of on-treatment Ki-67 was combined with pathologic tumor stage and ER status to develop the preoperative endocrine prognostic index (PEPI). A PEPI score of 0 (pT1/2N0, Ki-67 ≤ 2.7% and persistently expressed ER) was associated with such favorable long-term outcome after neoadjuvant endocrine therapy in the P024 trial (11) and IMPACT trial (10, 12) that chemotherapy was proposed to be unnecessary (13).

Recently, Ellis et al published long-term follow-up results of the ACOSOG Z1031 trial in which clinical decisions were based on the PEPI score(14). In ACOSOG Z1031 Cohort B the authors tested the hypothesis that Ki-67-based algorithms can also address the concern that patients who are poorly responsive to neoadjuvant endocrine therapy should ideally be identified early for triage to alternate treatment, such as neoadjuvant chemotherapy or immediate surgery. The authors also successfully identified a subgroup of patients, based on PEPI scores that could be safely spared from adjuvant chemotherapy. In this paper we describe the validation of Ki-67 cut-points relevant to neoadjuvant endocrine treatment monitoring and the development and validation of the Ki-67 clinical trial assay for prospective studies, used in ACOSOG Z1031 trial(14).

Methods

Database analysis for early Ki-67 cut point for early triage to alternate treatment

Published data on research use only (RUO) quantitative polymerase chain reaction (qPCR)-based assignments of PAM50 luminal subtype (A versus B) and RUO Ki-67 data from TMA analysis was made available from six hundred sixty seven tumors with clinical ER positive status from University of British Columbia. Of these tumors, 358 were classified as Luminal A and 309 as Luminal B (7). Published Ki-67

data and clinical outcomes from the IMPACT trial (12) and POL Trial (15, 16) were used for the development of cut-points for prospective validation.

Tumor samples for Ki-67 clinical trial assay development.

For training the scanner and image analysis-based Ki-67 quantification algorithm, 61 node positive samples from the P024 trial were examined. For assay validation for the early triage cut-point, core needle biopsies taken after 4 weeks of neoadjuvant endocrine therapy from 66 patients were accessed (15). For validation of the 2.7% cut-point required for the PEPI score, surgical specimens from 58 patients with pathological stage 1 or 2A tumors were available from a combination of the POL trial (15) and the P024 trial (11).

Ki-67 Assay methodology

The research use only (RUO) Ki-67 assay employed to stain the P024 and POL samples for combined survival analysis employed the SP6 monoclonal antibody (Neomarkers) on a Shandon Sequenza® Immunostainer using published methodology (13). For the CLIA clinical trial assay, 5 micron sections from POL and P024 trials were subjected to H&E and Ki-67 staining in the CLIA-certified Washington University AMP laboratory using the CONFIRM anti-Ki-67 (30-9) rabbit monoclonal primary antibody as a pre-diluted reagent on a Benchmark XT platform according to the manufacturer instructions (Ventana, Tucson, AZ). Tonsil was used as the assay control.

Ki-67 Scoring approaches

For visual point counting (VPC), photomicrographs of three randomly selected fields were taken at 40X with a background grid and color printed (more fields to achieve the minimal cell count). Each observer counted both the total number tumor cells and the number of Ki-67 positive cells that intersect with first grid line. This process is repeated on every third gridline. All the cells on the slide were counted if three fields could not be obtained however at least 200 total tumor cells were required. For Ki-67 image analysis of the CLIA clinical trial assay, slides were scanned with the iScan Coreo scanner (Ventana). The computer image was reviewed and “Areas of Interest” (AOI) were selected at 4X magnification using the following guidelines: 1) identify the largest AOI of representative clear invasive tumor; 2) exclude DCIS, vessels, lymphocytes; 3) avoid AOIs in peri-necrotic or necrotic areas; 4) identify at least 3 AOIs and a maximum of 10. The image analysis was performed using the FDA cleared VENTANA Companion Algorithm Ki-67 (30-9) and the VENTANA VIRTUOSO software (Roche).

Assessment of concordance

Two pathologists, blinded to each other's data and any data from earlier analyses of the samples, independently reviewed the Ki-67 slide scans and identified AOI for either image analysis or VPC methodology. Similarly blinded trained technicians generated the VPC Ki-67 percentage.

Statistical Analysis

Analysis of variance using a scatter-plot analysis was calculated using Pearsons' correlation and Spearman correlation coefficients. Two-pathologist concordance for the 2.7% and 10% cut-points were analyzed using four by four contingency table analysis, simple Kappa coefficients and percent positive and negative agreements. The prognostic effect of modified PEPI 0 (pT1/2, N0, Ki-67 ≤ 2.7%) vs. non-0 assignments based on the CLIA Ki-67 assay determined using the Kaplan–Meier method. The log-rank test was conducted to examine statistical significance. Similar analyses were performed to correlate survival outcomes of patients with early on treatment Ki-67 (>10% vs ≤ 10%) in the POL trial. Bland-Altman plots were generated to assess bias between pathologists.

Results

A Ki-67-based definition of poorly endocrine therapy responsive tumors for triage to alternate treatment

To develop a Ki-67-based approach for the early identification of non-responders within a month of starting treatment, we examined the interaction between baseline Ki-67 levels and a qPCR-PAM50-based definition of luminal A versus luminal B breast cancer using published data (17). Using ROC methodology, a 10% Ki-67 cut point of Ki-67 best served as a surrogate for the genomic luminal definitions in this data set (Figure 1). We therefore hypothesized that tumors with an early Ki-67 value above 10% despite endocrine therapy would be enriched for endocrine therapy resistant, luminal B-type tumors with a high relapse rate. This is supported by the early on-treatment data from the POL (15) and IMPACT (12) trials which indicated that Ki-67 levels > 10% predicted a higher level of Ki-67 in the surgical sample, a higher PEPI score, a smaller number of patients in the PEPI-0 group and worse RFS (14)

PEPI score validation and modification. Long-term outcomes from the POL trial provided an opportunity to further validate of the PEPI score. While the number of cases was modest, no relapses were observed in 10 patients with PEPI 0 tumors after a median follow up of 59 months (Figure 2A). We also developed a modified PEPI score that did not include ER status at surgery, because of clinical trial proposals that included the use of the estrogen receptor down-regulator fulvestrant, the use of which confounds the interpretation of ER levels after treatment initiation (18). In the P024, IMPACT and POL trials, patients with modified PEPI score of 0 were all ER+ (Allred score 3-8) because ER Allred score 0-2 post aromatase inhibitor

or tamoxifen treatment was associated with either a high Ki-67 or high tumor staging (or both) excluding these cases from PEPI-0 status without the need for information on ER. In the combined P024 trial/POL trial data, no relapses were observed in the 29 patients (19 pT1N0, 10 pT2N0) with modified PEPI-0 status (i.e. without scoring ER) during a median follow up of 62.5 months (Figure 2B).

Validation of visual point counting (VPC) for outcome prediction after neoadjuvant endocrine therapy

In previous analyses, VPC methodology was routinely used but this approach had not been formally assessed as part of a clinical trial assay. Available surgical tumor samples from pT1/2 N0 cases in the POL and P024 trials were therefore stained using the commercial 30-9 antibody assay in a CLIA certified laboratory. Stage 1 or 2A cases were chosen because a Ki-67 cut-point (CP) of ≤ 2.7% is the only factor that determines the modified PEPI score of 0. The REMARK sample flow chart for the duplicate study is provided in Figure 3A. Outcome predictions were reproducible, with no relapses observed for patients assigned modified PEPI 0 (Ki-67 ≤ 2.7%) status by either pathologist (Figure 3B). Analysis of Ki-67 as a continuous variable indicated that the Spearman Correlation Coefficient was 0.938 ($p< 0.0001$) (Figure S2A) and there was no trend for increased discordance across the range of Ki-67 values (Figure S2B). The positive CP agreement was 13/13 (100%). The negative agreement was 9/12 (0.75) (95% exact confidence limit: 0.428-0.945). Simple Kappa Coefficient was 0.7573 (95% Confidence limit: 0.5073; 1) (Table 1 and S1A).

Assessment of an image analysis approach for Ki-67 scoring

The performance of VPC, while technically adequate, is laborious and therefore not ideal for real time clinical reporting. We therefore considered a Ki-67 scoring approach using an FDA cleared scanner and image interpretation software to determine if these tools were appropriate. For training, the 30-9 antibody-based commercial assay was conducted on 61 surgical samples from patients with node positive disease in the P024 trial (Figure S1). The slides were scanned and then analyzed by two pathologists who independently reviewed the images and drew areas of interest (AOI) for Ki-67 scoring. In five instances, the algorithm did not accurately differentiate between benign and malignant cells. These cases were noteworthy for abundant lymphocyte infiltration, sparse tumor cells where tumor cells were streaming through the tissue with a large amount of intervening stroma, abundant marking of non-fascicular “plump” fibroblasts, or when the Ki-67 stain was generally diffuse and nuclear staining was faint. Excluding these cases, the Spearman Correlation Coefficient was 0.89 ($p<0.0001$) (Figure S3A). The Bland-Altman plot showed no bias in scoring between the two pathologists across the range of Ki-67 values (Figure S3B). The CP concordance was then analyzed. For the 2.7% cut point, the positive agreement was 29/30 (0.96) (95% exact confidence limit: 0.82-0.99). The negative agreement was 23/26 (0.88) (95% exact confidence limit: 0.69-0.97). The kappa coefficient was 0.85 (95% confidence limit: 0.71; 0.99). Using

the 10% cut-point, the positive agreement was 100%, and the negative agreement was 46/47 (0.97) (95% exact confidence limit: 0.88-1). The kappa coefficient was 0.93 (95% Confidence limit: 0.81; 1.0) (Table 1 and S1B). A “locked-down” scoring standard operating procedure (SOP) was generated that included an option to triage to VPC if the pathology was judged too complex for the scanner to differentiate benign from malignant cells (Figure 4).

Validation of combined imaging/VPC Ki-67 scoring SOP for the 2.7% Ki-67 cut point

To validate the combined imaging/VPC SOP for Ki-67 scoring, the CLIA assay stained slides used for the VPC assessment were scanned and independently assessed by two pathologists. The sample flow chart is shown in Figure 5A. Kaplan-Meier analysis by modified PEPI 0 is shown in Figure 5B for the two separate scoring exercises. Again, no relapses were observed in patients with modified PEPI 0 during the follow up using this scoring method from either pathologist. Continuous data analysis indicated that the Spearman Correlation Coefficient was 0.86 ($p<0.0001$) (Figure S4A). No scoring bias was observed across the scored range (Figure S4B). The percentage positive agreement between the two pathologists in scoring the 2.7% CP Ki-67 using the SOP was 0.87 (95% CI 0.61-0.98). The negative agreement was 0.88 (95% CI 0.65-0.98). Simple kappa coefficient was 0.76 (95% CI 0.54-0.98) (Table 1 and S1C).

Validation of combined imaging/VPC Ki-67 scoring SOP for the 10% Ki-67 cut point

To validate the combined imaging/VPC approach for the 10% cut point one-month biopsies from the POL trial were stained using the Ki-67 30-9 clinical trial assay, scanned and then independently reviewed for algorithm accuracy and independently scored by two pathologists. The REMARK sample flow chart is shown in Figure 6A. Concordant Kaplan-Meier analyses for the 10% cut point for two separate scoring exercises are shown in Figure 6B. The poor outcome for patients in the >10% category was reproducible. The Spearman Correlation Coefficient was 0.86 ($p<0.0001$) (Figure S5A). No scoring bias was observed across the scoring range (Figure S5B). The percentage positive agreement between the two pathologists in scoring the 10% CP Ki-67 using the SOP was 100%. The negative agreement was 93.6 (78.6-99.2). The kappa coefficient was 0.86(0.66-1) (Table 1 and S1D).

Discussion

We have developed an efficient and reproducible Ki-67 scoring system that was approved by the Clinical Trials Evaluation Program (CTEP) for NCI-supported neoadjuvant endocrine therapy trials. The combination of image analysis with triage to VPC, when deemed necessary, respects the finding that the image analysis

software does not always differentiate between certain types of normal and malignant cells. This approach also emphasizes the critical role of the pathologist in the review of the scanned images to determine the most appropriate scoring approach (image analysis or VPC) when the histology is complex. The sample flow charts illustrate that while pathologists may have different interpretations for the requirement for visual point counting, these differences do not strongly affect clinical outcome prediction. The VPC triage rate was, on average 17%, demonstrating that the image analysis approach can be used in the majority of cases, markedly reducing the need to conduct laborious VPC to a manageable number of cases. A weakness of our study is that the sample sets were denuded by earlier analyses and produced very modest sample sizes and therefore our analysis did not produce evidence for immediate clinical utility. However, the the Ki-67 clinical trial assay developed and described in this paper was further validated in ACOSOG Z1031A study. In that trial, with a median follow-up of 5.5 years, this Ki67 methodology was able to identify a subgroup of patients with PEPI score =0 (Ki-67≤2.7%, T1/2, N0) that were safely managed without adjuvant chemotherapy. Among patients with PEPI=0 score that were managed without chemotherapy, only 4 out of 119 presented with a relapse during follow-up. The triage rate to VPC in the Z1031A trial was 6%, even lower than what we found in the POL and P024 sample sets.

An issue not addressed in the scoring algorithm proposed herein concerns cases where the Ki-67 staining is not uniform – our VPC or image analysis approach requires random fields. We consider a Ki-67 heterogeneity-agnostic approach equivalent to genomic approaches that also do not clearly respect tissue heterogeneity. While analysis of heterogeneity, or “Ki-67 hot spot” analysis, should be pursued, this is a complex problem that will require the development of a “hot-spot” definition that can be shown to drive outcome more effectively than an analysis of all the tumor cells in the section.

Another point of controversy is the Ki-67 cut point as a surrogate for luminal A versus luminal B breast cancer. In our current analysis 10% has the best operating characteristics while an earlier publication on a different data set, using similar methodology suggested 14% (7), which suggests a narrow range of values for this purpose. From the perspective of this paper, the 10% cut-point was more conservative and serves the purpose of early identification of patients with luminal B-type tumors with endocrine therapy resistance characteristics well. The rapid onset of advanced disease for patients with Ki-67 > 10% despite aromatase inhibitor therapy (see Figure 6B for example) underscores the importance of developing a robust clinical trial strategy for this high risk population.

When we submitted our Ki-67 clinical trial assay to the FDA they ruled the proposed treatment algorithms as “no significant risk” because Ki-67 analysis actually reduces the risk of under-treatment. This conclusion was based on the analysis of chemotherapy use according to PEPI score shows that when medical oncologists rely on pathological stage alone after neoadjuvant endocrine therapy most patients with low stage do not receive chemotherapy. Combined analysis of the P024, IMPACT and POL trials showed that only 8% of patients with pathological stage 1 or 2A disease received adjuvant chemotherapy (Table S2). Thus, the FDA considered that knowledge of the Ki-67 value in the pathological specimen reduced the risk of

under-treatment for patients with low pathological stage tumors but aggressive biological characteristics (high on-treatment Ki-67).

Even though ASCO still does not support Ki67 in its clinical guidelines, a recent editorial acknowledges our team's efforts as "an important step in the direction of clinical respectability for Ki67 as a useful breast cancer prognosticator"(19). The next necessary step is already being taken as the Ki-67 clinical trial assay we described in this paper is being prospectively validated in the ALTERNATE trial (NCT01953588).

Acknowledgements: We would like to thank Dr Lisa McShane for guidance on the development plan for the Ki-67 assay, Dr Mitch Dowsett and Dr Roger A'Hern for analysis of the IMPACT data and Dr Torsten Nielsen for analysis of the University of British Columbia tamoxifen series. We would also like to thank the FDA for their review on the necessity of an IDE application for Ki-67 analysis in CTEP sponsored neoadjuvant endocrine therapy trials. This work was supported by grants to Dr. Matthew Ellis from the AVON Foundation, R01 CA095614, the Barnes-Jewish Foundation, the Breast Cancer Research Foundation and Novartis (for biomarker studies on the P024 trial). The tissue procurement core was supported by NCI P50 CA68438. Dr R. Goncalves was supported by the AVON foundation.

Figure Legends

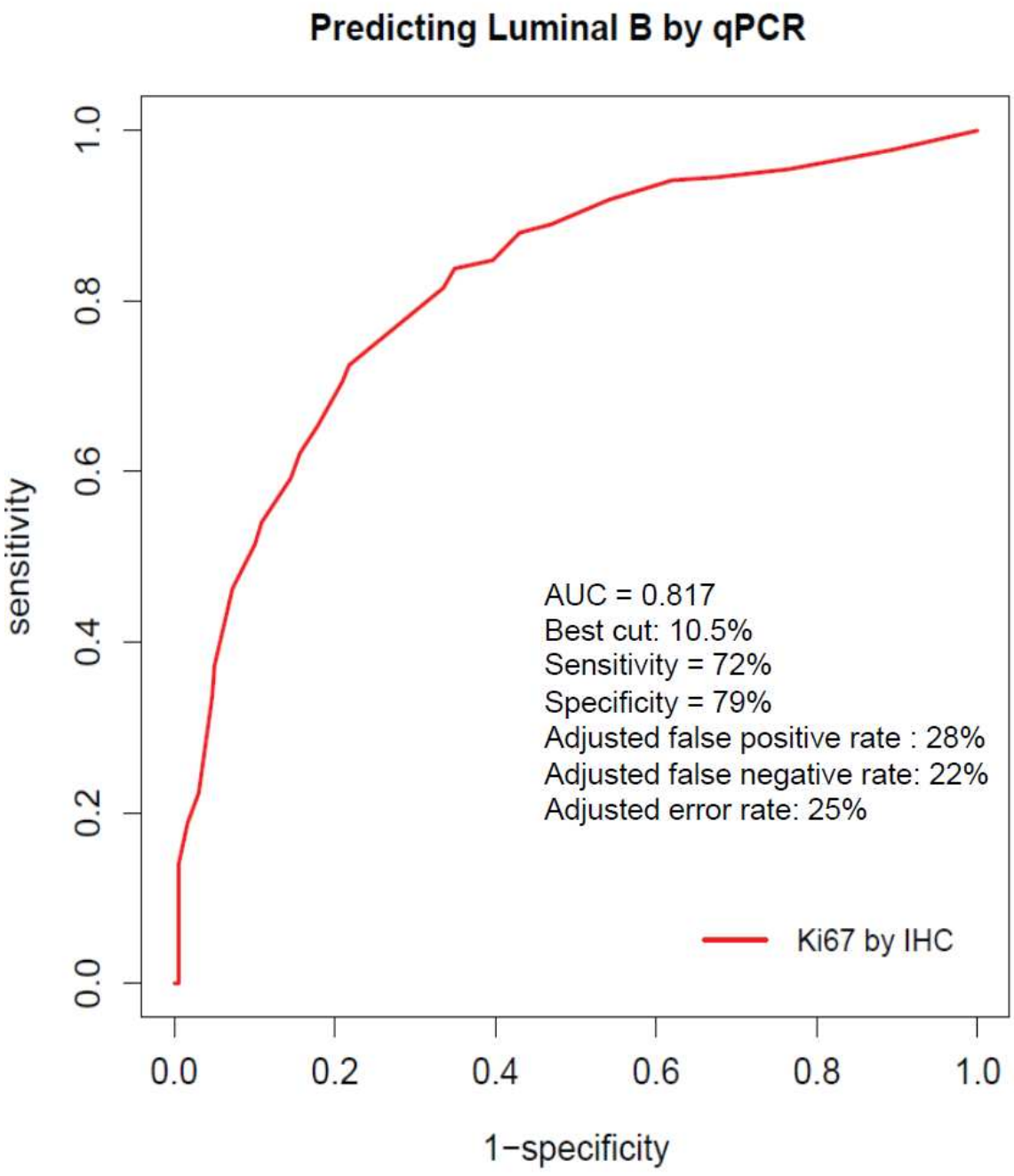

Figure 1: ROC curve to determine the best Ki-67 cut-point to differentiate Luminal A breast cancer from Luminal B breast cancer based on a PAM50 qPCR RUO assay

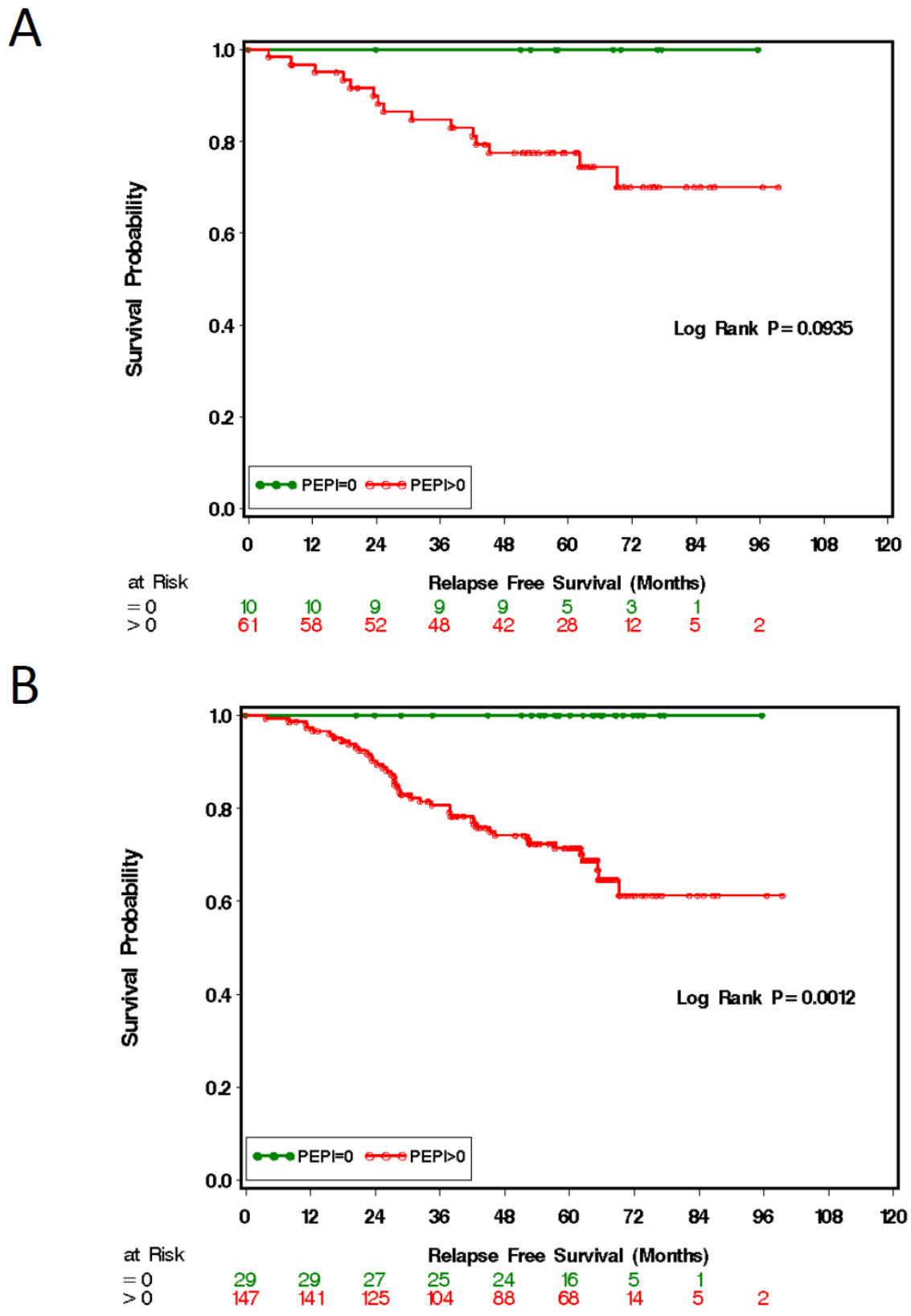

Figure 2: Kaplan-Meier curves showing relapse-free survival in the POL trial (A) and in the combined data from the POL/P024 trials using an RUO assay and VPC scoring methodology (B). (PEPI: Preoperative endocrine prognostic index)

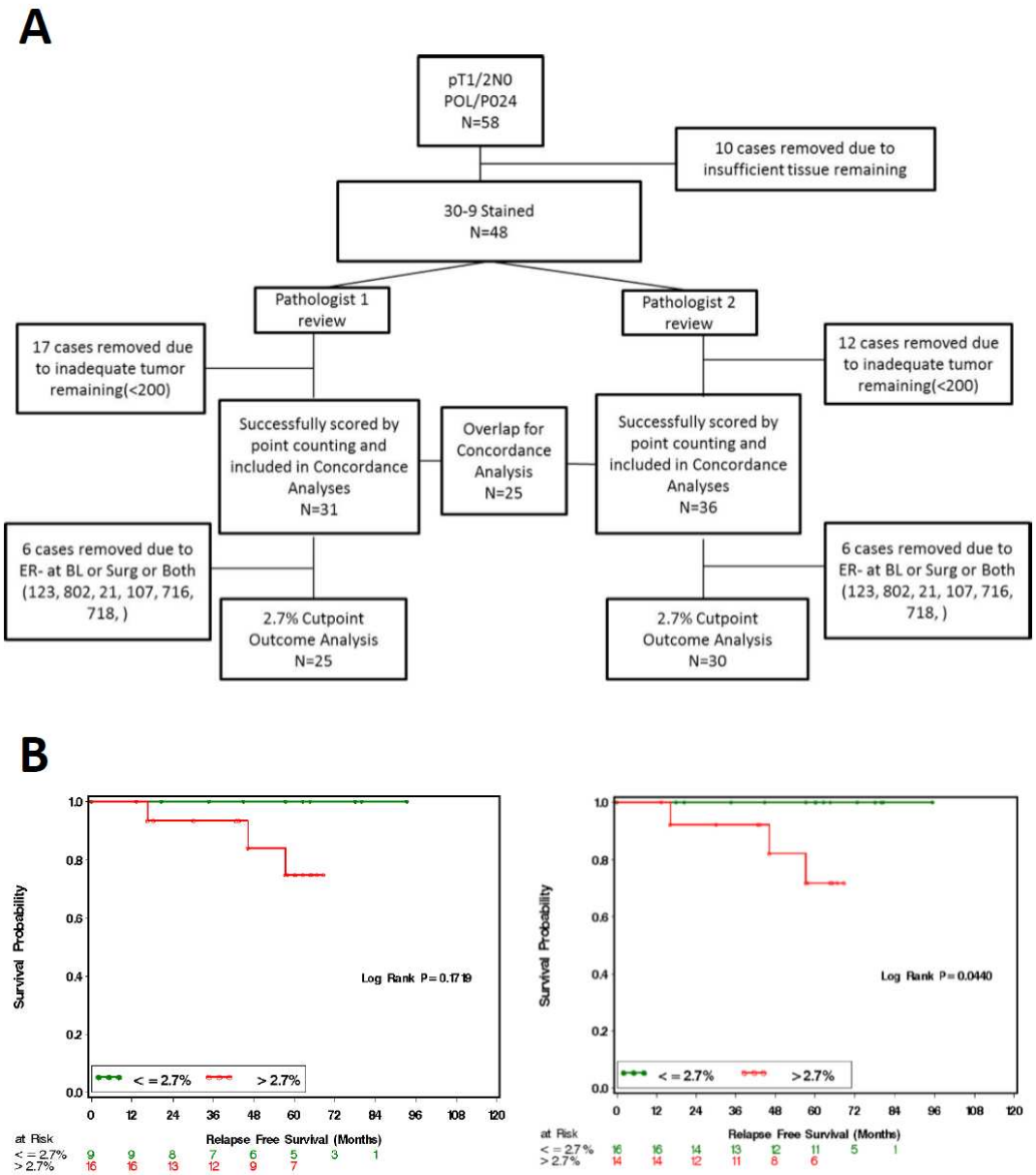

Figure 3: (A) REMARK diagram showing sample flow through the study for validation of the visual point counting technique. (B) Kaplan-Meier curves from two independent pathologists demonstrating relapse-free survival according to Ki-67 score > 2.7% or ≤ 2.7%

Description of the Ki-67 Scoring SOP for 30-9 stained slides

1. Pathologist review of scanned image to identify cases for point counting using the following criteria:

A. Abundant intermixed lymphocytes within the invasive tumor.
B. Sparse invasive tumor.
C. Diffusely infiltrative tumor with no clear consolidation (ie, streaming invasive tumor with a large amount of intervening stroma).
D. Any situation leading to abundant marking of stromal elements (ie, non-fascicular "plump" fibroblasts).
E. Any situation where an abundant amount of stained cells are not being included in the analysis such as general diffuse and faint nuclear staining.

2. AOI selection for image analysis for cases fit for image analysis:

A. At 4x, pick the largest AOI of representative clear invasive tumor and draw the FOV so as to minimize non-invasive tumor (ie, stromal elements).
B. Exclude areas of artifact such as crushed cells, smeared/ blurred staining, and tissue folds
C. Exclude DCIS, vessels, lymphocytes (obviously to a degree).
D. No AOIs in peri-necrotic areas.
E. Pick at least 3 AOIs this way and a maximum of 10.
F. For each FOV drawn, ensure the computer is appropriately marking relevant tumor cells versus non-relevant tumor cells. If not, the case should be flagged for point counting.

3. Manual Point counting for those triaged for point counting

A. Select at least 3 random fields under 40x images
B. Color print on photomicrographs with a background grid
C. Counted the total tumor cells and the number of Ki67 positive cells that intersect with first grid line. Repeated on every third gridline.
D. All the cells on the slide were counted if three images could not be obtained, so that at least 200 total tumor cells were counted.

4. The percent positive Ki67 value was obtained using the following equation: [number of Ki67 positive cells/total number of tumor cells] x 100%.

Figure 4: Standard operating procedure (SOP) for Ki-67 scoring with the aid of an image scanner and the Companion Algorithm image analysis software

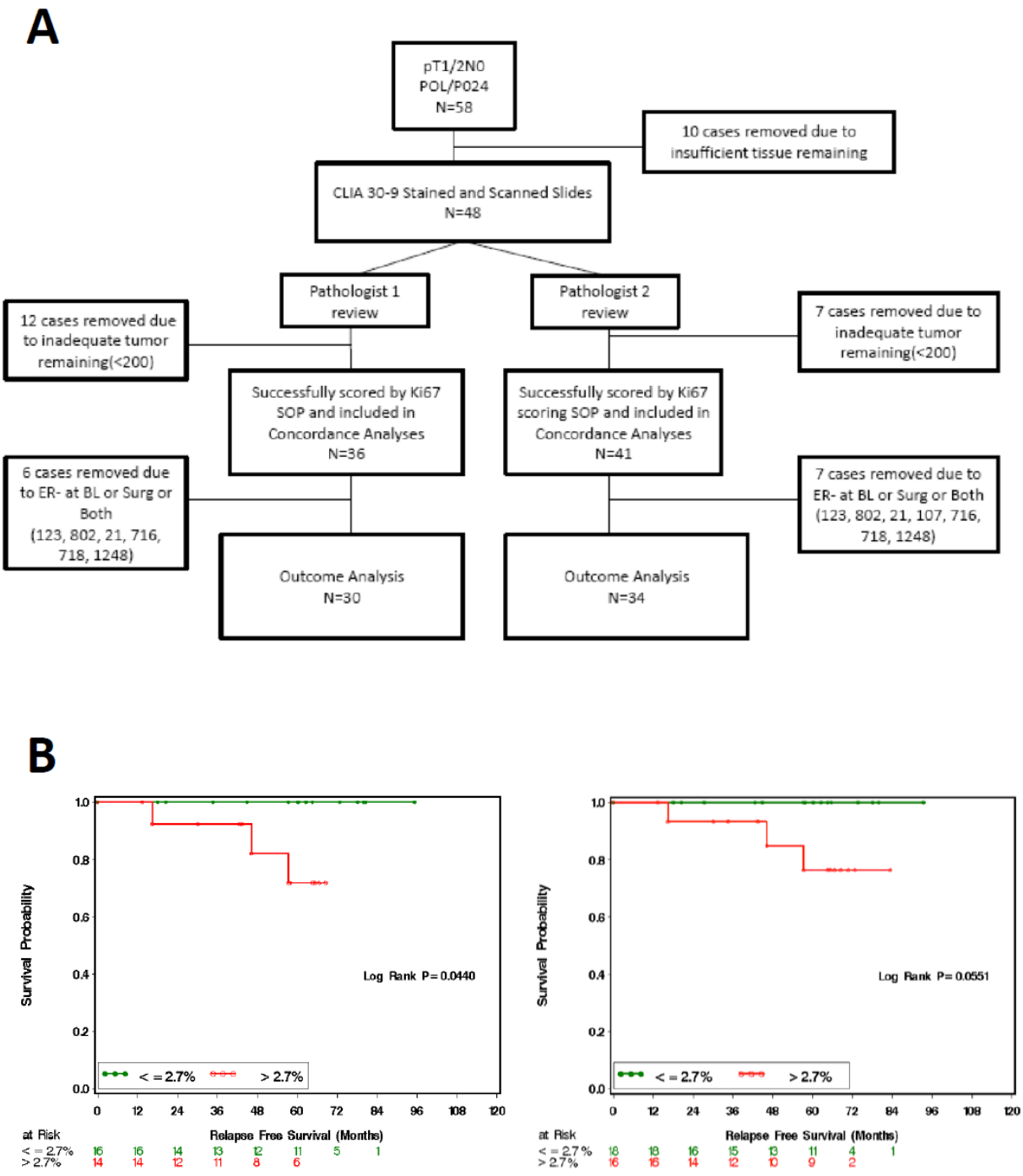

Figure 5: (A) REMARK diagram showing patient flow through the study for validation of the standard operating procedure for Ki-67 scoring. (B) Kaplan-Meier curves from two independent pathologists demonstrating relapse-free survival according to Ki-67 score ≤ 2.7% or > 2.7%

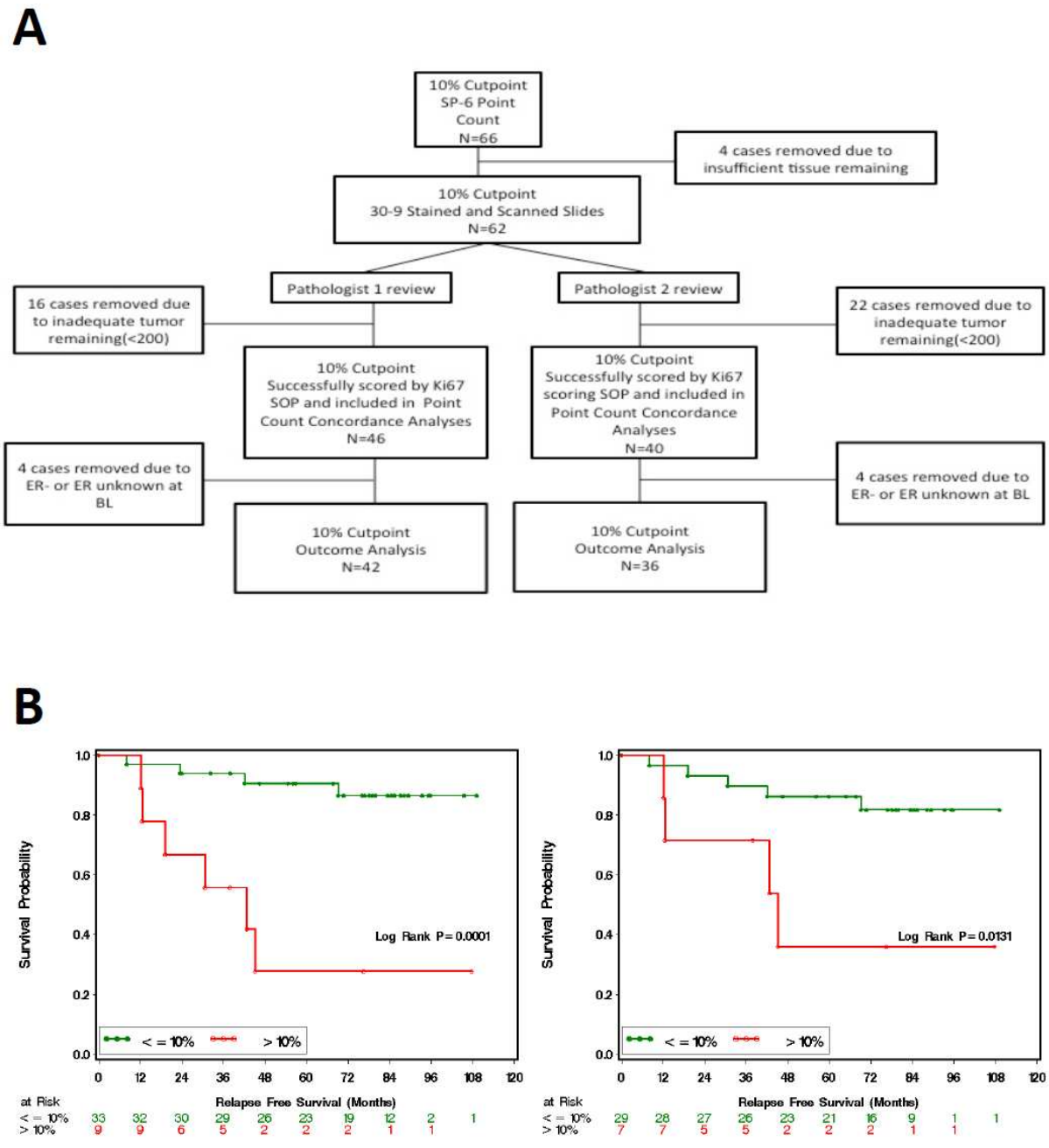

Figure 6: (A) REMARK diagram showing patient flow through the study for validation of the standard operating procedure for Ki-67 scoring. (B) Kaplan-Meier curves from two independent pathologists demonstrating relapse-free survival according to Ki-67 score ≤10% or >10% (B)

Table 01: Summary of Ki-67 scoring agreement statistics according to the sample sets and different counting methods used

Sample set	Ki-67 Cut-point	Counting method	Percent Positive Agreement (95%CI)	Percent Negative Agreement (95%CI)	Kappa coefficient (95%CI)
Validation set	2.7%	Visual Point Counting	100	75 (42.8;94.5)	0.76 (0.51-1)
Training set	2.7%	Virtuoso Software	96.7 (82.8-99.9)	88.5 (69.9; 97.6)	0.85(0.72;0.99)
	10%	Virtuoso Software	100	97.9 (88.7;1)	0.94 (0.81;1)
Validation set	2.7%	Ki-67 SOP	87.5 (61.7;98.5)	88.9 (65.3;98.6)	0.76(0.55;0.98)
	10%	KI-67 SOP	100	93.6 (78.6;99.2)	0.86(0.66;1)

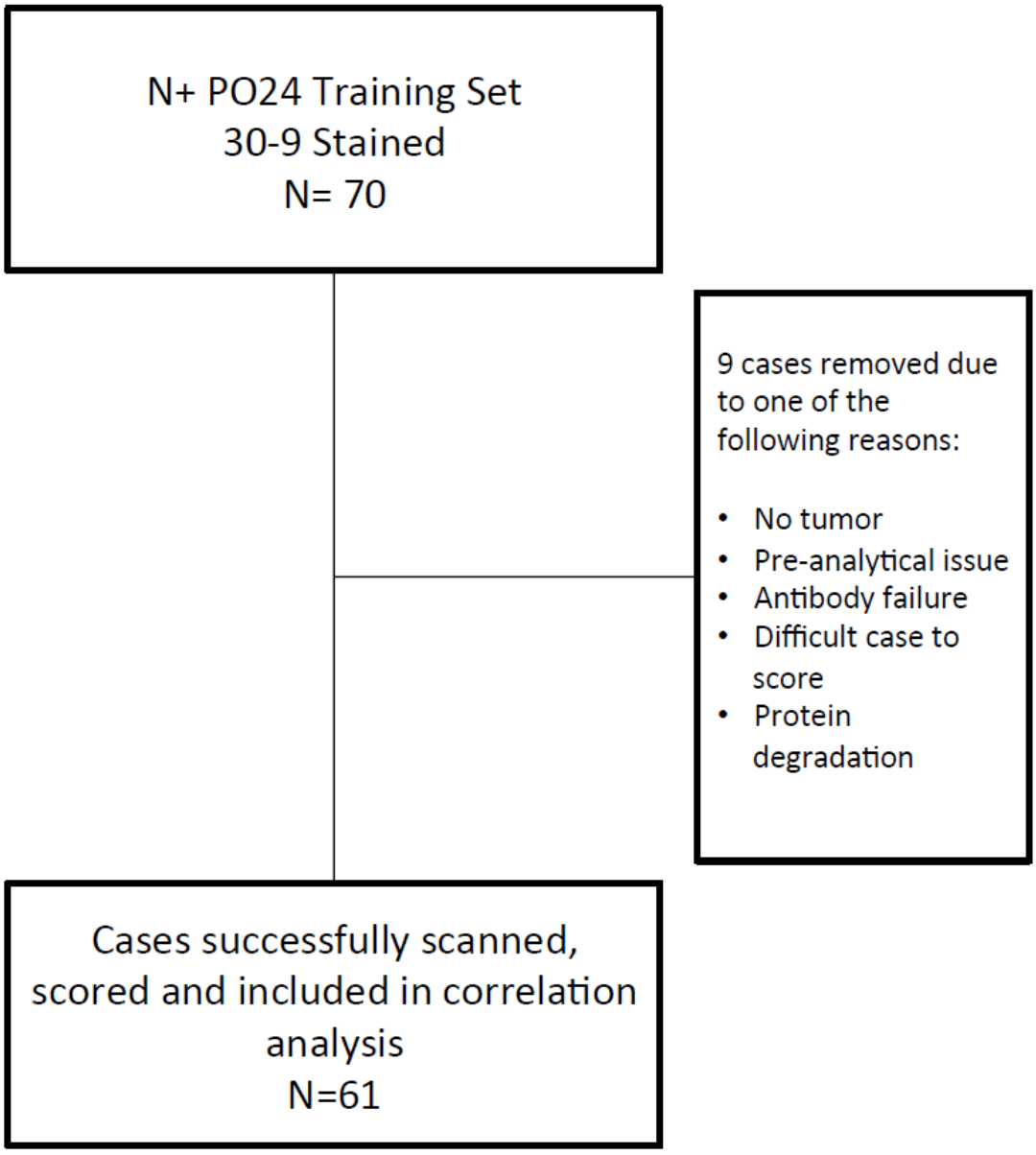

Figure S1: Diagram demonstrating the patient flow for the training set to test the use of 30-9 stained samples and the Virtuoso software

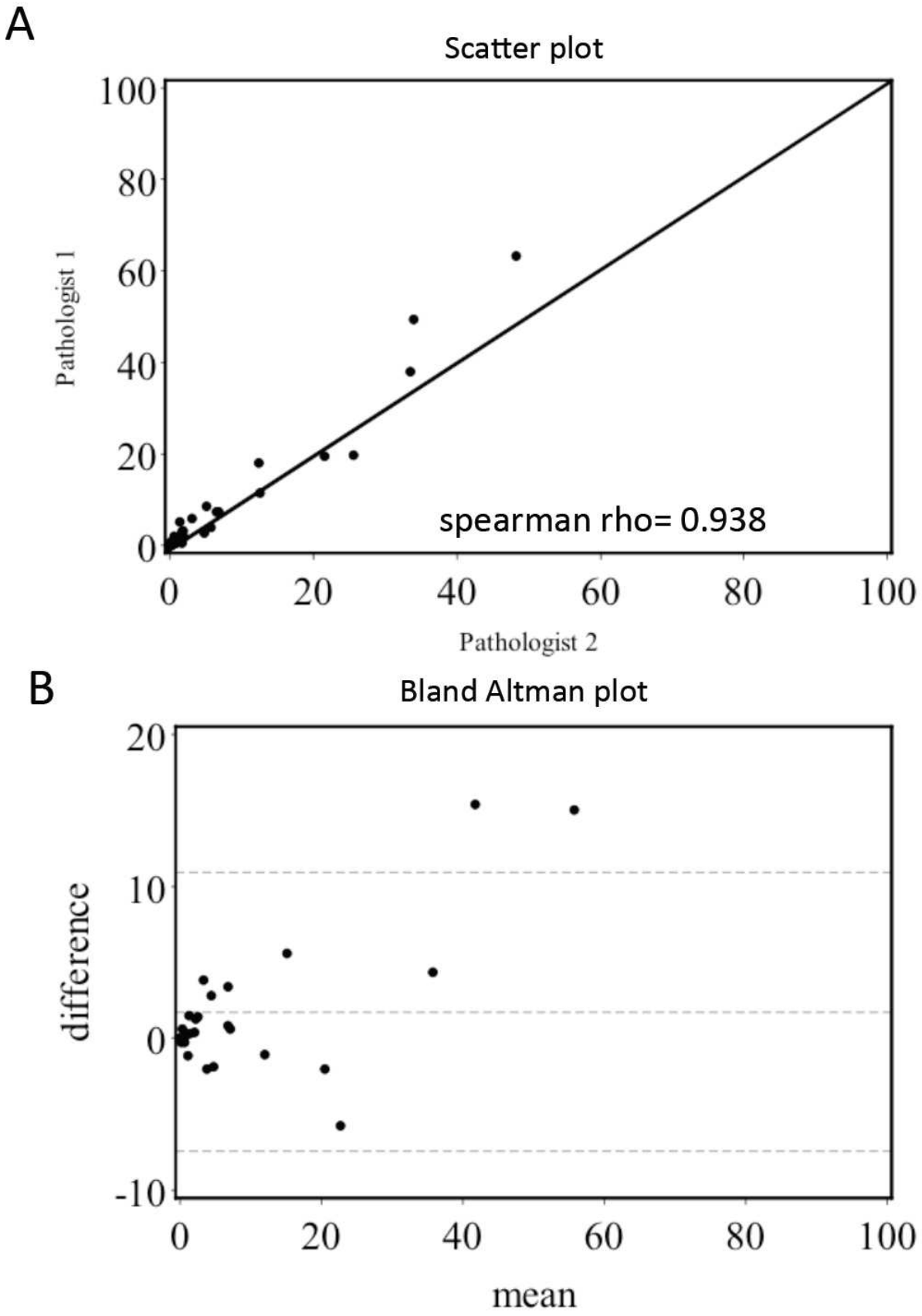

Figure S2: Scatter plot (A) and Bland Altman plot (B) for the concordance analysis of two pathologists using the visual point counting technique for Ki-67 scoring in the validation set

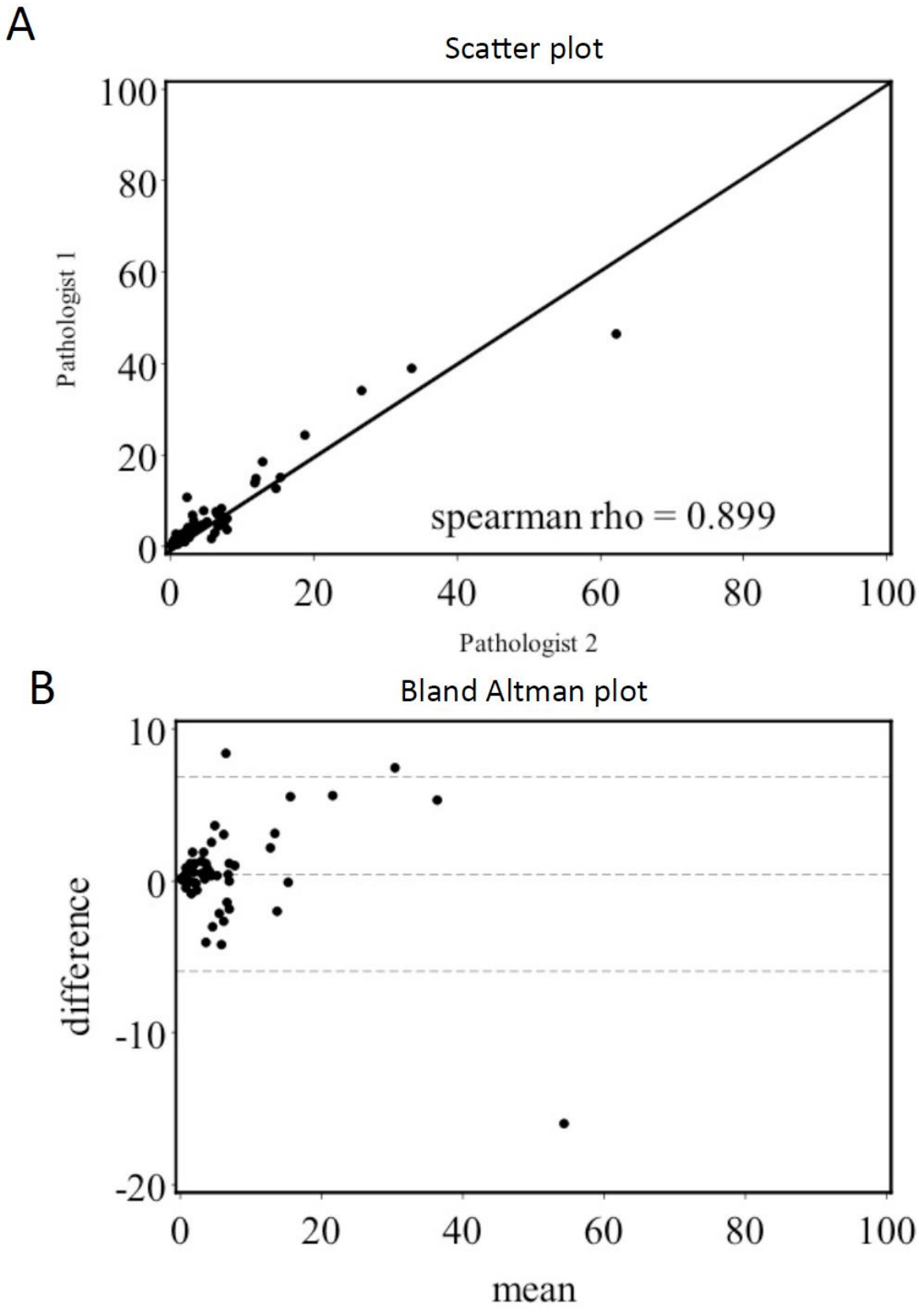

Figure S3: Scatter plot (A) and Bland Altman plot (B) for the concordance analysis of two pathologists using the image analysis software for Ki-67 scoring in the training set

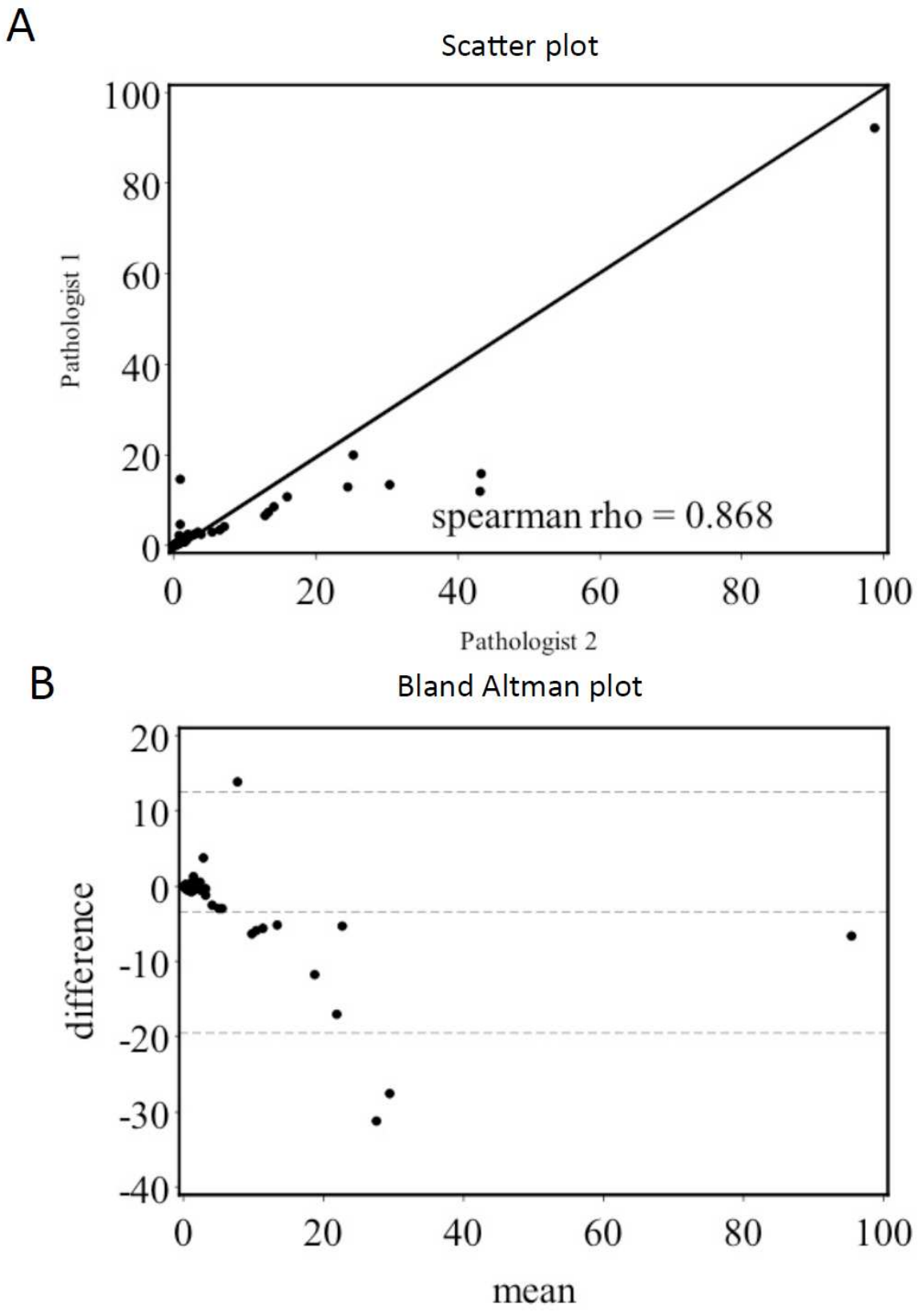

Figure S4: Scatter plot (A) and Bland Altman plot (B) for the concordance analysis of two pathologists using the combined imaging/VPC Ki-67 scoring SOP in the 2.7% validation set

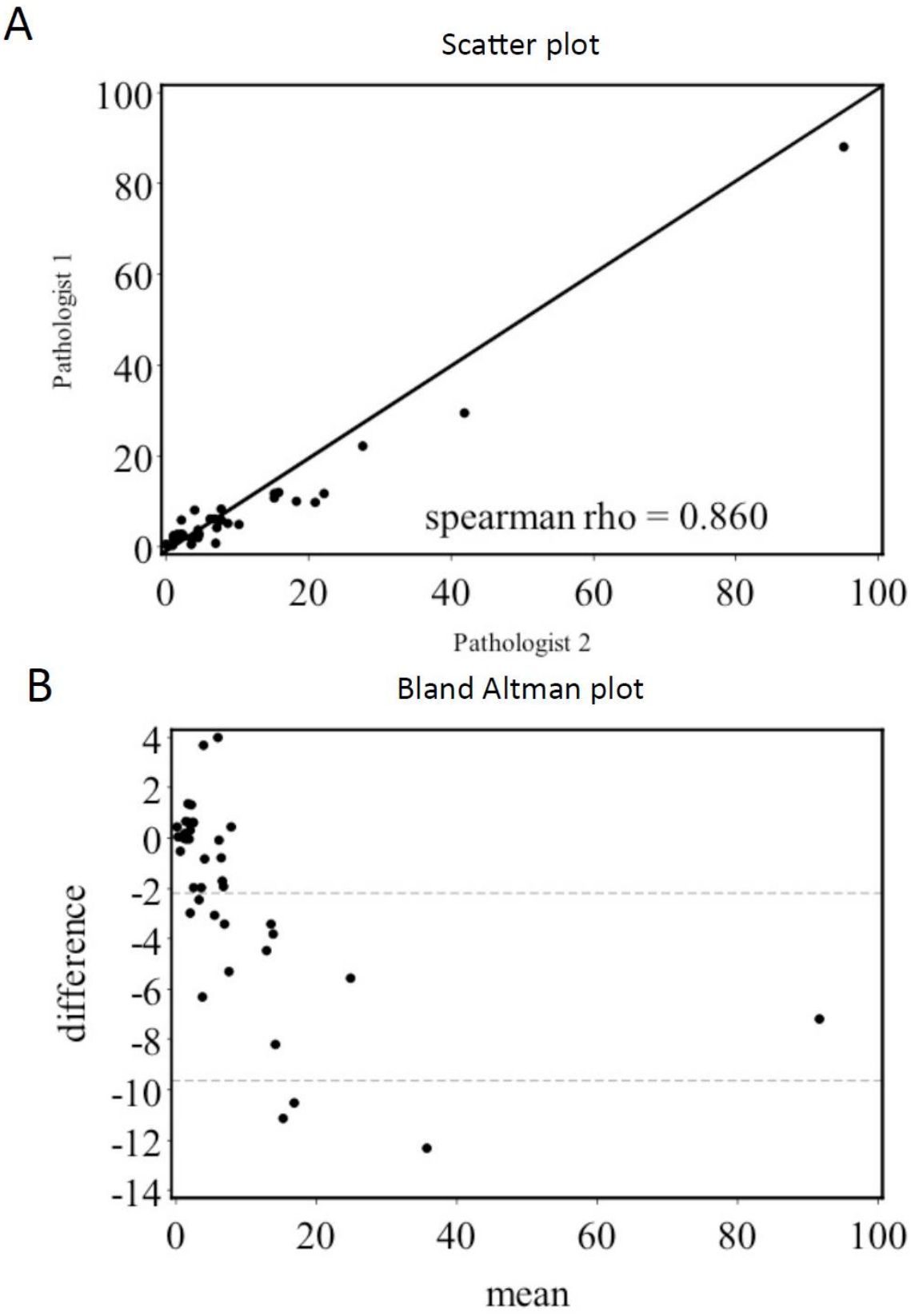

Figure S5: Scatter plot (A) and Bland Altman plot (B) for the concordance analysis of two pathologists using the combined imaging/VPC Ki-67 scoring SOP in the 10% validation set

Supplementary Table 1: (A) Two pathologist concordance in Ki-67 visual point counting of CLIA 30-9 stained Breast Cancer surgical samples from pT1/2N0 cohort of P024/POL trial. (B) Two pathologist concordance in Ki-67 image analysis of CLIA 30-9 stained Breast Cancer surgical samples from the node positive cohort of P024 trial. C) Two pathologist concordance for the combined imaging and VPC Ki-67 SOP scoring of the CLIA 30-9 stained pT1/2N0 cohort of P024/POL trials. (D) Two pathologist concordance for the combined imaging and VPC Ki-67 SOP scoring of the CLIA 30-9 stained 4-week biopsy in POL trial.

A)

Two Pathologist Concordance in Ki67 visual point counting of CLIA 30-9 stained Breast Cancer surgical samples from pT1/2N0 cohort of P024/POL trial

2.7% cut point	Pathologist 2		
Pathologist 1	> 2.7%	≤ 2.7%	Total
> 2.7%	13	3	16
≤ 2.7%	0	9	9
Total	13	12	25

B)

Two Pathologist Concordance in Ki67 image analysis of CLIA 30-9 stained Breast Cancer surgical samples from the Node Positive Cohort of P024 trial.

2.7% cut point	Pathologist 2		
Pathologist 1	>2.7%	≤ 2.7%	Total
>2.7%	29	3	32
≤ 2.7%	1	23	24
Total	30	26	56

10% cut point	Pathologist 2		
Pathologist 1	> 10%	≤ 10%	Total
>10%	9	1	10
≤ 10%	0	46	46
Total	9	47	56

C)

Two Pathologist Concordance for the final Ki67 SOP scoring of the CLIA 30-9 stained pT1/2N0 cohort of P024/POL trial

2.7% cut point	Pathologist 2		
Pathologist 1	> 2.7%	≤ 2.7%	Total
> 2.7%	14	2	16
≤ 2.7%	2	16	18
Total	16	18	34

D)

Two Pathologist Concordance for the Ki67 SOP scoring of the CLIA 30-9 stained 4-week biopsy in POL trial

10% cut point	Pathologist 2		
Pathologist 1	> 10%	≤ 10%	Total
> 10%	8	2	10
≤ 10%	0	29	29
Total	8	31	39

Supplementary Table 2: Record of chemotherapy usage in the POL, IMPACT and P024 neoadjuvant endocrine therapy trials according to PEPI score (Ki-67 values were determined retrospectively, therefore the decision to use chemotherapy in these trials was based on standard clinical factors.

Chemotherapy administration in the P024, IMPACT and POL trials				
P024\PEPI	0	1-3	4+	Total
Chemotherapy	5 (12%)	24 (37%)	28 (54%)	57
Total	41	65	52	158

IMPACT\PEPI	0	1-3	4+	Total
Chemotherapy	1 (3%)	21 (22%)	26 (35%)	48
Total	31	97	75	203

POL\PEPI	0	1-3	4+	Total
Chemotherapy	1 (9%)	16 (57%)	24 (67%)	41
Total	11	28	36	75

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Artigo 2

Decision Letter (November 9, 2016)

To: Matthew J Ellis <mjellis@bcm.edu>, Candyce.Cummings@bcm.edu
From: aislinn.boyter@asco.org
Subject: JCO/2016/694406 - Manuscript Decision
Cc: "Vera J. Suman" <suman@mayo.edu>, Jeremy Hoog <jhoog@dom.wustl.edu>, Rodrigo Goncalves <rodrigomed38@gmail.com>, Souzan Sanati <ssanati@path.wustl.edu>, Chad J Creighton <creighto@bcm.edu>, Katherine DeSchryver <kdspath1@yahoo.com>, Erica Crouch <crouch@wustl.edu>, Amy Brink <abrink@wustl.edu>, Mark Watson <watsonm@pathology.wustl.edu>, Jingquin Luo <luor@wudosis.wustl.edu>, Yu Tao <taoy@wudosis.wustl.edu>, Michael Barnes <michael.barnes.mb1@roche.com>, Mitchell Dowsett <mitch.dowsett@icr.ac.uk>, Tom Budd <buddg@ccf.org>, Eric Winer <eric_winer@dfci.harvard.edu>, Paula Silverman <paula.silverman@uhhs.com>, Laura Esserman <laura.esserman@ucsfmedctr.org>, Lisa Carey <lisa_carey@med.unc.edu>, Cynthia X Ma <cynthiaxma@wustl.edu>, Gary Unzeitig <drgwu@laredobreastcare.com>, Timothy Pluard <tpluard@saint-lukes.org>, Pat Whitworth <patwhitworth@gmail.com>, Gildy Babiera <gvbabiera@mdanderson.org>, J Michael Guenther <joseph.guenther@stelizabeth.com>, Zoneddy Dayao <zdayao@salud.unm.edu>, David Ota <david.ota@duke.edu>, Marilyn Leitch <marilyn.leitch@utsouthwestern.edu>, John A Olson <jolson@smail.umaryland.edu>, "D. Craig Allred" <stldca@gmail.com>, Kelly Hunt <khunt@mdanderson.org>

Dear Dr. Ellis,

I am pleased to inform you that your manuscript, "Ki67 proliferation index as a tool for chemotherapy decisions during and after neoadjuvant aromatase inhibitor treatment for breast cancer: Results from the ACOSOG Z1031 Trial (Alliance).," has been accepted for publication in Journal of Clinical Oncology. See below for important information concerning proofs, National Institutes of Health grantees (if applicable), and embargo.

Additional manuscript preparation will be completed by Editorial within approximately 7 business days. Your manuscript will then be sent to Production for copy editing and composition; the manuscript proof will be available for review by the corresponding author in approximately 4 to 6 weeks after acceptance. (See below for important details regarding your proof.) If you have any questions regarding manuscript production, please contact Production Manager Catherine Forrest (jcocorrections@asco.org; 571-483-1692). Please refer to your manuscript number in your message. We also encourage you to alert your public affairs office as soon as possible regarding the pending

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Thank you for giving us the opportunity to publish your work.

Sincerely,

Pamela Goodwin, MD, MSc

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Ki67 proliferation index as a tool for chemotherapy decisions during and after neoadjuvant aromatase inhibitor treatment for breast cancer: Results from the ACOSOG Z1031 Trial (Alliance)

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Purpose: To determine the pathologic complete response (pCR) rate in estrogen receptor positive (ER+) primary breast cancer triaged to chemotherapy when the Ki67 level was >10% after 2-4 weeks of neoadjuvant aromatase inhibitor (AI) therapy. A second objective was to examine risk of relapse using the Ki67-based Preoperative Endocrine Therapy Prognostic Index (PEPI).

Patients and Methods: Z1031A enrolled postmenopausal women with stage II/III ER+ (Allred 6 to 8) breast cancer (BC) whose treatment was randomized to neoadjuvant AI therapy with anastrozole, exemestane or letrozole. For Z1031B the protocol was amended to include a tumor Ki67 determination after 2-4 weeks of AI. If the Ki67 was >10% patients were switched to neoadjuvant chemotherapy. A pCR rate of >20% was the predefined efficacy threshold. In patients who completed neoadjuvant AI, stratified Cox modeling was used to assess whether time to recurrence differed by PEPI=0 score (T1/2, N0, Ki67<2.7%, ER Allred >2) versus PEPI >0 disease.

Results: Only two of the 35 patients on Z1031B switched to neoadjuvant chemotherapy experienced a pCR (5.7%, 95%CI: 0.7-19.1%). After 5.5 years of median follow-up, 4 of the 109 (3.7%) patients with aPEPI=0 score relapsed versus 49 of 341 (14.4%) PEPI>0 patients, recurrence hazard ratio (PEPI=0/PEPI>0) = 0.27 (p=0.014; 95%CI: 0.092-0.764).

Conclusions: Chemotherapy efficacy lower than expected in ER+ tumors exhibiting AI-resistant proliferation. The optimal therapy for these patients should be further investigated. For patients with PEPI=0 disease the relapse risk over 5 years was only 3.6% without chemotherapy supporting the study of adjuvant endocrine monotherapy in this group. These Ki67 and PEPI triage approaches is being definitively studied in the ALTERNATE trial (NCT01953588).

Introduction

For post-menopausal women with clinical stage II/III estrogen receptor positive (ER+) breast cancer neoadjuvant aromatase inhibition (AI) is an under-utilized and low-toxicity alternative to chemotherapy for increasing breast conservation rates[1]. A barrier to greater adoption of neoadjuvant AI is high response variability. We therefore postulated that an early switch from AI to neoadjuvant chemotherapy could produce better clinical outcomes for tumors that were responding poorly to AI. Conversely, adjuvant AI alone may be sufficient to prevent relapse in tumors that are highly responsive to neoadjuvant AI.

Pathological complete response (pCR) after systemic chemotherapy remains a controversial clinical trial endpoint [2,3] and alternatives are needed, particularly in ER+ HER2- disease where pCR rates are low. Data from a neoadjuvant study comparing letrozole and tamoxifen in post-menopausal women with ER+ breast cancer (P024[4-6]) was previously used to generate the "Preoperative Endocrine Prognostic Index" (PEPI)[7]. PEPI requires pathologic stage (tumor size and nodal status) in addition to Ki67 levels and Allred ER score measured on the surgical specimen (with surgery conducted during uninterrupted endocrine therapy). Patients with a PEPI score of 0 (pT1/2, pN0, Ki67 ≤ 2.7%, Allred score >2) from the P024 trial were found to have a very low risk of relapse. Similar findings were observed in the neoadjuvant IMPACT trial[2] (Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen) but no subsequent validation efforts have been reported

The American College of Surgeons Oncology Group (ACOSOG) Z1031A randomized phase II clinical trial was designed to determine which AI (anastrozole, letrozole or exemestane) should be recommended for future testing against chemotherapy in the neoadjuvant setting (ACOSOG is now a part of the Alliance for Clinical Trials in Oncology). The major initial finding from Z1031A was that half of the patients who were considered candidates for mastectomy or inoperable prior to neoadjuvant AI therapy had successful breast conserving surgery[8]. When the enrollment to Z1031A was complete, an amendment was introduced (Z1031B) that triaged patients with tumors exhibiting a Ki67 > 10% in a tumor biopsy 2-4 weeks after starting AI to standard chemotherapy. The hypothesis being tested was that the pCR rate would be at least 20% in this AI resistant population. Herein, we report the pCR results from Z1031B as well as the time to recurrence by PEPI status among all Z1031 patients who completed neoadjuvant AI treatment.

Methods

Establishment of an early Ki67 cut point for triage to chemotherapy. An on-treatment Ki67 threshold for switching from neoadjuvant AI therapy to neoadjuvant chemotherapy was established using data from the Preoperative Letrozole study (POL)[9] and the IMPACT trial[10]. A Ki67 value of more than 10% at one month in the POL studies was associated with a higher PEPI score (P=0.01), a smaller number of patients in the PEPI-0 group (P=0.08) and worse RFS (P=0.0016). Similarly the IMPACT data confirmed that a two week Ki67 >10% predicted a higher PEPI score (P=0.001), smaller numbers of patients in the PEPI-0 group (P= 0.004) and worse

RFS (P=0.008) (Supplementary Table 1 and Supplementary Figures 1A and 1B)). Combining these studies revealed only one PEPI-0 case among 51 patients with a 2 to 4 weeks Ki67 value of greater than 10%. Thus, according to the PEPI model, patients with a Ki67 value of 10% at 2 to 4 weeks had less than a 2% chance of a favorable PEPI score that would allow them to safely avoid chemotherapy under current guidelines.

Patient Eligibility. Eligible patients were postmenopausal with clinical stage T2-T4c, N0-3, M0 invasive breast cancer. Additional criteria have been previously described[8]. This study was supported by the Clinical Trials Support Unit and approved by the institutional review boards of all participating institutions. Each participant signed an IRB-approved, protocol-specific informed consent in accordance with federal and institutional guidelines.

Treatment Schema. Patients enrolled onto Z1031B underwent a core breast biopsy for Ki67 determination after 2 weeks of AI therapy. If the Ki67 was ≤10% the patient continued AI therapy for another 12-14 weeks and then proceeded to surgery. Women whose two-week Ki67 level was > 10% were offered either a NCCN approved neoadjuvant chemotherapy regimen or surgery at the discretion of providers/patients. If the biopsy core contained insufficient tumor to perform the Ki67 assay patients could elect to be re-biopsied at 4 weeks or continue on AI therapy. If severe treatment-related toxicity was reported or the patient refused further AI therapy, surgery was recommended. Within 14 days of registration, patients underwent a complete physical examination with tumor assessment. Every 4 weeks, patients underwent a physical examination, toxicity assessment, and tumor assessment using February 2000 WHO criteria. If tumor progression was suspected, ultrasound or mammogram was required and neoadjuvant AI was discontinued if progression was confirmed. Blood and biopsy specimens for correlative studies were collected at baseline, 2-4 week after the start of neo-adjuvant AI treatment, upon discontinuation of neo-adjuvant treatment and at surgery. For patients with PEPI=0 disease, management without chemotherapy was recommended but not mandatory in order to determine the acceptability of this recommendation.

Aromatase inhibitor Treatment. Before the release of the Z1031A results[8], eligible patients were randomized to 16-18 weeks of neo-adjuvant AI with exemestane 25 mg daily, letrozole 2.5 mg daily, or anastrozole 1 mg daily. After the release of the data, patients could choose either letrozole or anastrozole treatment.

Statistical Considerations. The primary endpoint for Z1031B was the pCR rate among the women who after 2 weeks of neoadjuvant AI therapy had a tumor Ki67 level of >10% and switched to neoadjuvant chemotherapy. pCR was defined as histologic evidence of no invasive tumor cells in the surgical breast specimen or ipsilateral lymph nodes. A one-stage phase II clinical trial design was chosen to assess the pCR rate. With a sample size of 35 and a one-sided alpha=0.10, a one sample binomial test of proportions would have a 90% chance of declaring success with a pCR rate of at least 20% compared to the null hypothesis that the pCR was 5% (at least 4 pCRs were needed to conclude pCR rate ≥ 20%). A 90% binomial confidence interval for the true pCR was also constructed. Based on the IMPACT study, it was estimated that 235 eligible women would need to enroll to obtain 35 women with a 2-week Ki67 > 10% willing to switch to neoadjuvant chemotherapy.

The long-term PEPI outcome study cohort excluded women from both Z1031A and Z1031B who withdrew consent not having started neoadjuvant AI, had metastases or bilateral invasive breast cancer at registration, failed to undergo surgery, had alternative therapy prior to surgery, had radiographic confirmed disease progression or new primary disease during neoadjuvant AI, failed to failed to have sentinel lymph node or axillary lymph node dissection or lacked sufficient tissue to obtain a Ki67 or Allred score. In addition, Z1031B patients with 2 or 4 week Ki67 > 10% who remained on AI were excluded. Time to breast cancer recurrence (TBCR) was defined as the time from surgery to first local, regional, or distant disease recurrence. Patients without documented disease recurrence were censored at the date of their last disease evaluation. TBCR was estimated using the Kaplan-Meier method[11] Stratified Cox modeling (with cohort and adjuvant chemotherapy use as strata) was used to assess whether TBCR differed with respect to PEPI 0 status[12]. The Alliance Statistics and Data Center conducted data collection and statistical analyses. Data were locked on January 11, 2016.

Ki-67 CLIA assay for Z1031A and Z1031B. For both Z1031 cohorts a Ki67 clinical trial assay was performed at the CAP/CLIA-certified Washington University AMP laboratory using the CONFIRM anti-Ki-67 (30-9) rabbit monoclonal primary antibody as a pre-diluted reagent on a Benchmark XT platform according to the manufacturer instructions (Ventana, Tucson, AZ). Tonsil was used as the assay positive control.

Ki-67 Quantification approaches. A single experienced pathologist (DCA) conducted the real-time Ki67 scoring for Z1031B. If the estimated rate was very low (<2.7%), or very high (>10%), a whole slide estimate was conducted. If the score was between 2.7% and 10% point counting was conducted using an ocular grid, at least 3 high power fields with a minimum of 100 cells scored. Retrospective analysis of Z1031A used the iScan Coreo scanner (Ventana) with the Companion Algorithm Ki-67 (30-9) software. The imaging approach required 3-10 areas of interest be selected at 4X magnification excluding DCIS, vessels, lymphocytes and avoiding peri-necrotic or necrotic areas. The image analysis result was reviewed to ensure the software was correctly differentiating between benign and malignant cells and if not, the case was triaged to visual point counting (VPC). The VPC approach required color photomicrographs with a background grid taken at 40X of at least three fields selected based on invasive tumor content and the quality of the histology, not on Ki67 staining pattern to obtain tumor cell count of at least 200. The scorer counted the total number of tumor cells and the number of Ki-67 positive cells that intersected with first grid line and every third gridline thereafter.

Gene Expression Analysis to study cell cycle regulated genes.

RNA preparation and Agilent 44K gene array analysis approaches were carried out as previously described[2]. The microarray contained probes for 720 of the 874 genes previously identified as having periodic expression in the cell division cycle of HeLa cells (Supplemental Table 2)[13]. Gene expression levels in each tumor were normalized to the number of standard deviations from the median expression value across all the tumors. A multigene proliferation score (MGPS) for a tumor was the average normalized expression of the 772 genes for that tumor analysis[14].

Results

Z1031B Patient Cohort

From October 1 2009, to November 15, 2011, 245 patients were enrolled into Z1031B. At week 2, 165 women (69.9%) had a Ki67 value of ≤10%; 49 women (20.8%) had a Ki67 value >10% and 22 women (9.3%) had insufficient tumor to make a Ki67 determination. Patient and disease characteristics of these 236 women are presented in Table 1 and the CONSORT diagram in Figure 1.

Neoadjuvant chemotherapy efficacy for ER+ tumors exhibiting a 2 to 4 week Ki67 value of greater than 10% after starting aromatase inhibition. Among the 49 patients whose week 2 Ki67 was >10%, 35 patients switched to neoadjuvant chemotherapy; three patients continued with AI, eight patients went directly to surgery, two patients went to surgery after a re-biopsy at week 4 and again found their Ki67 to be >10% and 1 patient pursued treatment outside of this study. Twenty-five of the 35 (71.4%) had an anthracycline-containing regimen (Table 2). Six patients (17.1%) failed to complete their planned course of chemotherapy due to intolerability. There were two (5.7%, 95%CI: 0.7-19.1%) pathologic complete responses (pCR) among these 35 patients. A pCR occurred in a 55 year-old woman with cT2N0 ductal breast cancer with a 2 weeks Ki67 of 80.0% treated with AC + docetaxel. The second pCR was in a 59 year-old woman with cT3N1 ductal breast cancer with a 2 week Ki67of 31.7% subsequently treated with docetaxel + gemcitabine + bevacizumab followed by AC + bevacizumab.

Neoadjuvant outcomes for Z1031B patients whose week 2 to 4 Ki67 was less than or equal to 10%. There were 187 patients whose 2-week Ki67 value was either ≤10% (165) or not determined due to insufficient tumor in the biopsy specimen (22). One of 187 patients whose week 2 Ki67 value was ≤10% chose to go directly to surgery rather than continue on AI. Of 22 patients whose 2-week Ki67 value was not obtained, all chose to remain on AI either after a re-biopsy at week 4 Ki67 of ≤ 10% (6 patients) or after forgoing a re-biopsy at 4 weeks (16 patients) (Figure 1 for exact disposition of all patients). Among the remaining 177 patients, pathological evaluation revealed no residual disease in the breast or lymph nodes in 3 patients, in only the breast in 92 patients, and in both the breast and lymph nodes in 82 patients (Table 3). The pCR rate among the 186 women who completed AI was therefore 1.6% (95% CI: 0.3 to 4.6%). PEPI scores were: 0 in 64 patients; 1-7 in 109 patients; not determined in 13 patients due to progression during neoadjuvant AI therapy (2 patients); lack of Allred score or Ki67 from surgical specimen (4 patients) or failure to undergo surgery for reasons other than progression (7 patients.) Thus, the PEPI 0 rate was 34.4% (95% CI: 27.6- 41.7%).

Adjuvant chemotherapy decisions for patients with a PEPI=0. Management without adjuvant chemotherapy was the preferred protocol approach for patients in the PEPI=0 category. This occurred in 57 of 64 PEPI=0 patients (89%). In contrast, 45 (41.2%) of 109 patients with a PEPI>0 received adjuvant chemotherapy.

Long-term outcomes for Z1031 patients according to PEPI status. Clinical outcomes after surgery were examined in 287 Z1031A patients and 173 Z1031B patients who completed neoadjuvant AI (REMARK Figure 2). Overall 119 (25.9%)

tumors were categorized as PEPI=0. Adjuvant chemotherapy was administered in 18 of 119 PEPI=0 cases (15.1%) and 162 of 341 (47.5%) PEPI>0 patients. Adjuvant radiation was administered in 80 of 119 PEPI=0 cases (67.2%) and 242 of 341 PEPI>0 cases (71.0%). At data lock, all patients have been followed until death or a median of 5.5 years post-surgery with 365 patients alive without a disease progression, 21 alive with disease recurrence, 32 died following disease recurrences; 42 patients died of: 1) a second cancer (6 patients); 2) non-cancer causes (28 patients) or 3) unknown causes (8 pts). A total of 49 patients with PEPI>0 disease experienced recurrence local (7), regional (2), distant (39) and loco-regional/distant (1) and 4 patients with PEPI=0 disease (all distant). The hazard of breast cancer recurrence for PEPI=0 cases relative to the PEPI>0 cases was 0.27 (p=0.014; 95%CI: 0.092-0.764) when stratifying by cohort and known adjuvant chemotherapy use. Kaplan Meier plots of the time to breast cancer recurrence by PEPI=0 versus PEPI>0 status are presented in Figure 3A for the combined cohort and in Figure 3B for those patients who did not receive adjuvant chemotherapy. Since the Ki67 assay approach in Z1031A is being tested prospectively in the ALTERNATE trial, the PEPI-0 outcome for Z1031A cohort is shown separately (Figure 3C).

Prognosis for Z1031B patients according to Ki67 at 2 to 4 weeks.

The 35 patients who were triaged to chemotherapy have been followed at least one year post-surgery or to death. At the time of the data lock, there were 24 patients alive without a disease progression, 6 were alive with disease progression, two had died from breast cancer and three had died due to non-cancer causes. With a median follow-up of 4.4 years post registration, the risk of relapse was increased for those with 2-4 week Ki67 > 10% (log rank P=0.004) (Figure 3D).

Gene expression analysis and treatment-induced tumor proliferation status. The low pCR rate to chemotherapy in Z1031B prompted a further investigation of tumor proliferation status. Paired high tumor content frozen tumor samples prior to and after 2 or 4 weeks of AI were available in 109 of the 245 patients and were used for mRNA expression profiling (REMARK, Figure 4A). Proliferation status was determined using a non-commercial multi-gene proliferation score (MGPS)[14]. Pairwise analysis demonstrated mRNA levels for ER, PgR and Ki67 and MGPS values were markedly suppressed with treatment (Wilcoxon signed rank P=>0.001). Box plots comparing the MGPS scores at baseline and 2 weeks are illustrated in Figure 4C. The MGPS scores were higher at both baseline and after 2 weeks in the cohort with 2 week Ki67 values of >10% compared with ≤10% (Figure 4C, $p < 0.001$). The Spearman Correlation coefficient at 2 to 4 weeks between Ki67 values and the MGPS was 0.49 (Figure 4D).

Discussion

The Preoperative Endocrine Prognostic Index (PEPI) is a distinct prognostic approach for ER+ breast cancer that depends on tumor features after neoadjuvant endocrine therapy. PEPI integrates residual disease burden and the cell cycle (Ki67) response providing a simple approach to deescalating adjuvant treatment after neoadjuvant AI for patients in the PEPI=0 category. These patients had a risk of

relapse of 3% with a median follow up of approximately 5 years are therefore unlikely to benefit from adjuvant chemotherapy. A weakness of the study is that the relapse risk estimate is based on only 119 cases, thus PEPI validation efforts should continue.

Concerns regarding the variability in Ki67 analysis have been discussed extensively[15]. The imaging analysis approach to Ki67 estimation used for the Z1031A cohort is promising (Figure 3C) but conclusive data using this methodology awaits the results of the ALTERNATE trial (NCT01953588).

The low pCR rate for Z1031B patients who switched to neoadjuvant chemotherapy contradicts the hypothesis that AI resistant proliferation in ER rich tumors is associated with enhanced chemotherapy response. The muted chemotherapy response was unlikely to be due to failure of the Ki67 assay to capture highly proliferative tumors since an independent examination of tumor cell cycle activity with a multi-gene proliferation score indicated significantly higher expression of cell cycle dependent genes when the Ki67 was >10% at 2 to 4 weeks (Figure 4C and 4D). Low chemotherapy responsiveness could reflect the postmenopausal status of the patient cohort[16], the high ER content of tumors eligible for neoadjuvant endocrine therapy[17,18] or the use of endocrine therapy before neoadjuvant chemotherapy. However, the latter hypothesis seems unlikely because, unlike prior studies that raised this concern[19], endocrine therapy was not administered concurrently with chemotherapy.

Our data provides further support for an assessment of prognosis in ER+ breast cancer based on post-neoadjuvant endocrine therapy tumor characteristics. Triaging patients to neoadjuvant chemotherapy based on failure to suppress Ki67 is feasible but highlights the relative chemotherapy resistant-nature of strongly ER+ yet AI-resistant disease in postmenopausal women and the early relapse risk faced by patients in this category in all three studies where this question can be examined (Figure 3D, supplementary Figures 1A and 1B). The development of new treatment options for intrinsically AI resistant disease will likely depend on new insights into the molecular basis for primary endocrine therapy resistance[20,21].

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Figure Legends

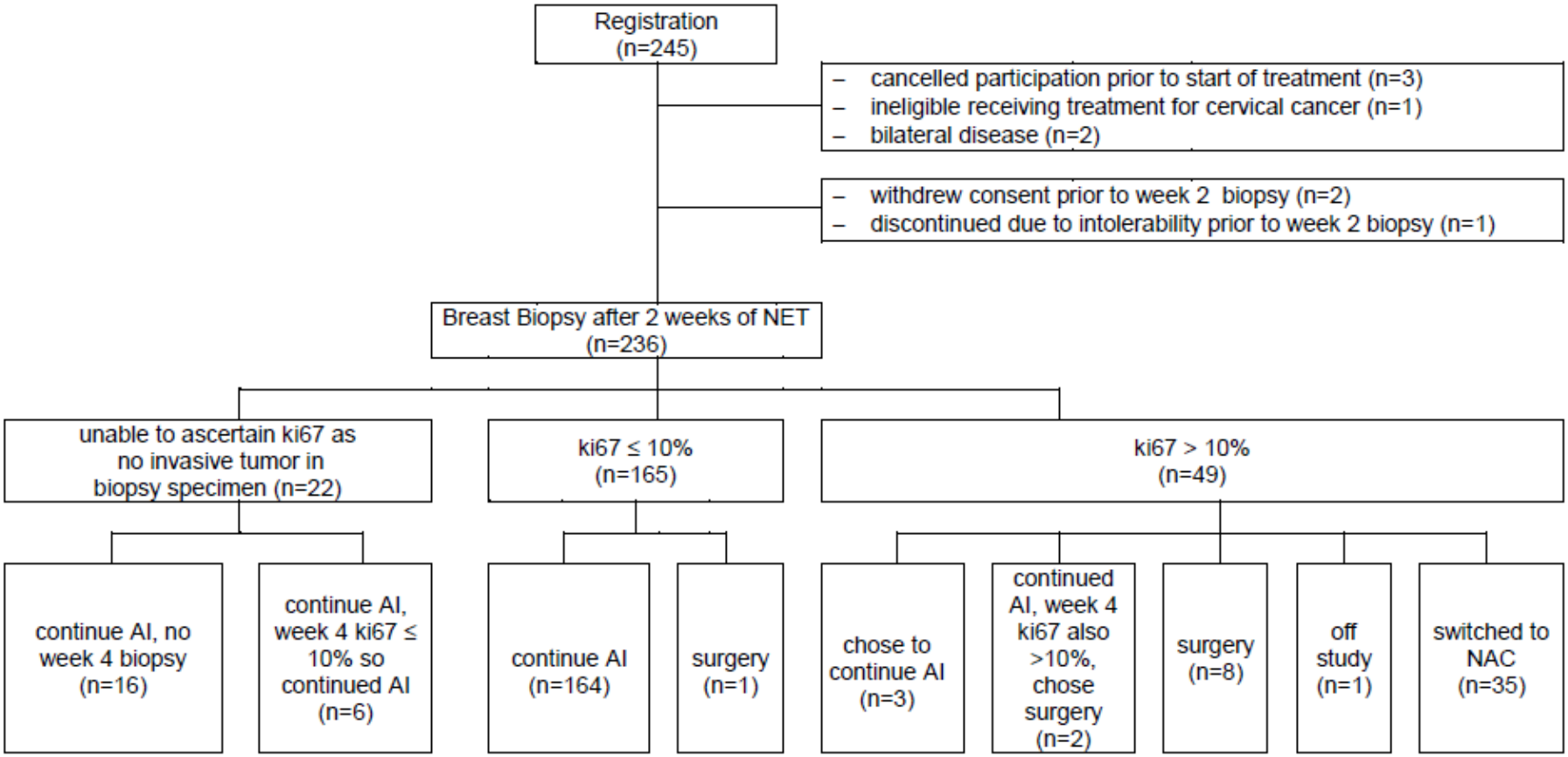
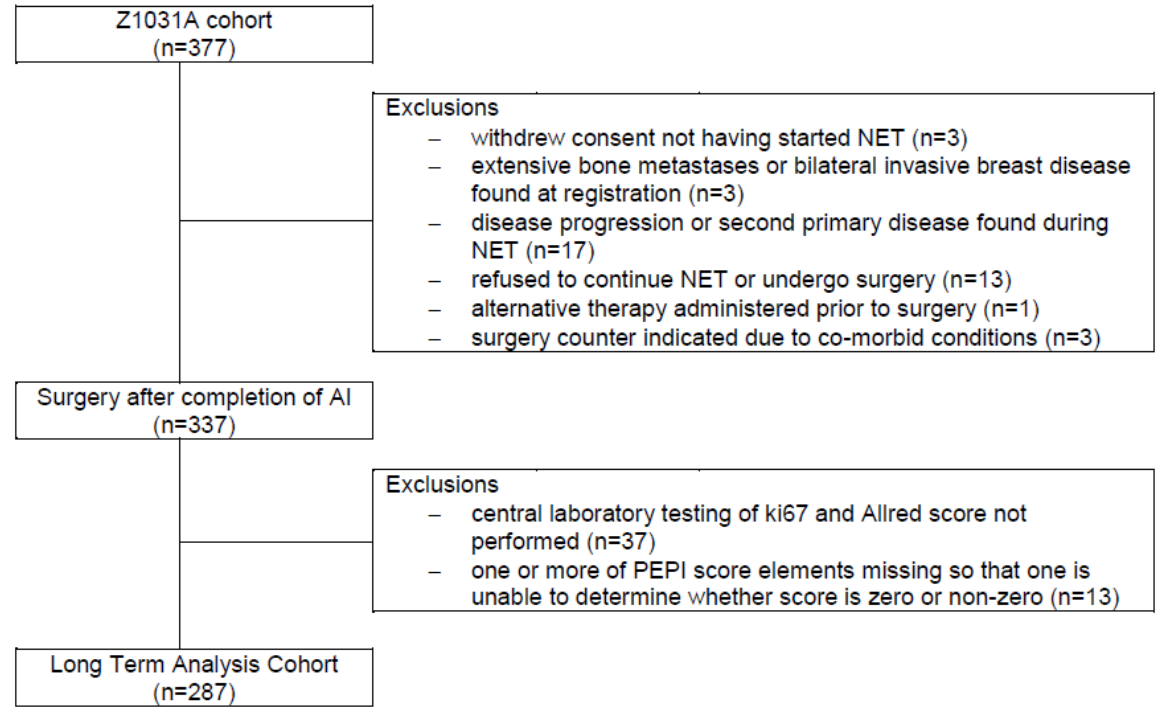


Figure 1: Consort diagram for Z1031B



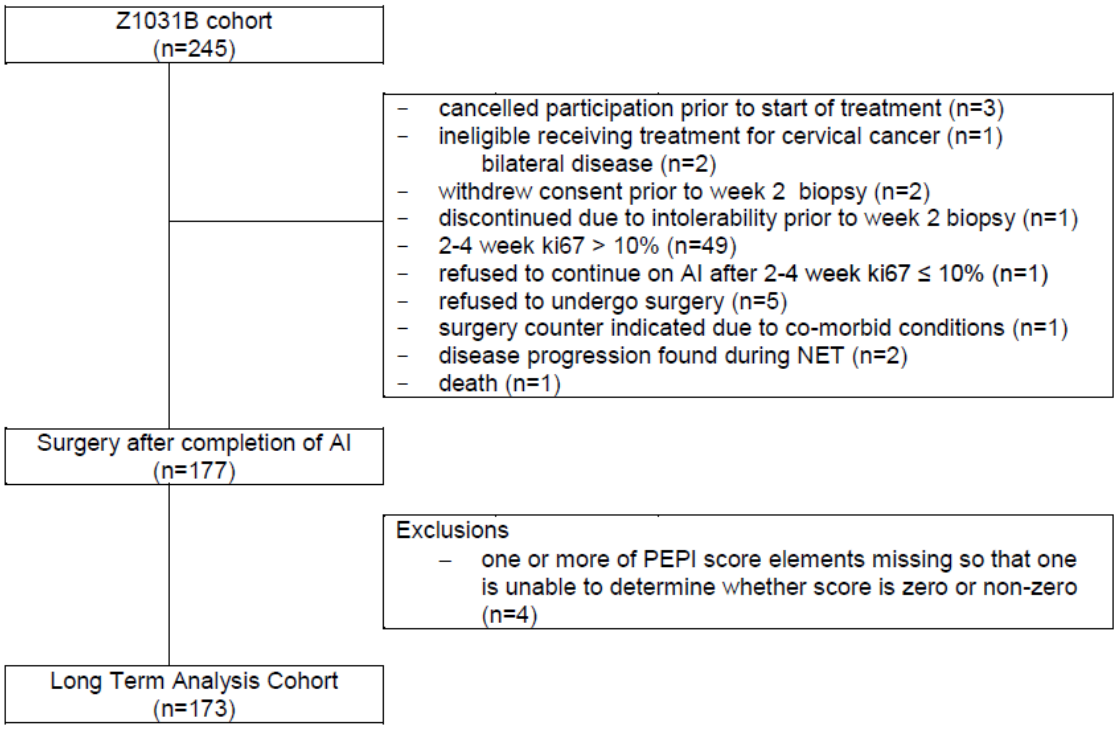


Figure 2. Remark Diagrams for the long-term outcome analysis by PEPI score for Z1031A patients (panel A) and for Z1031B patients (panel B)

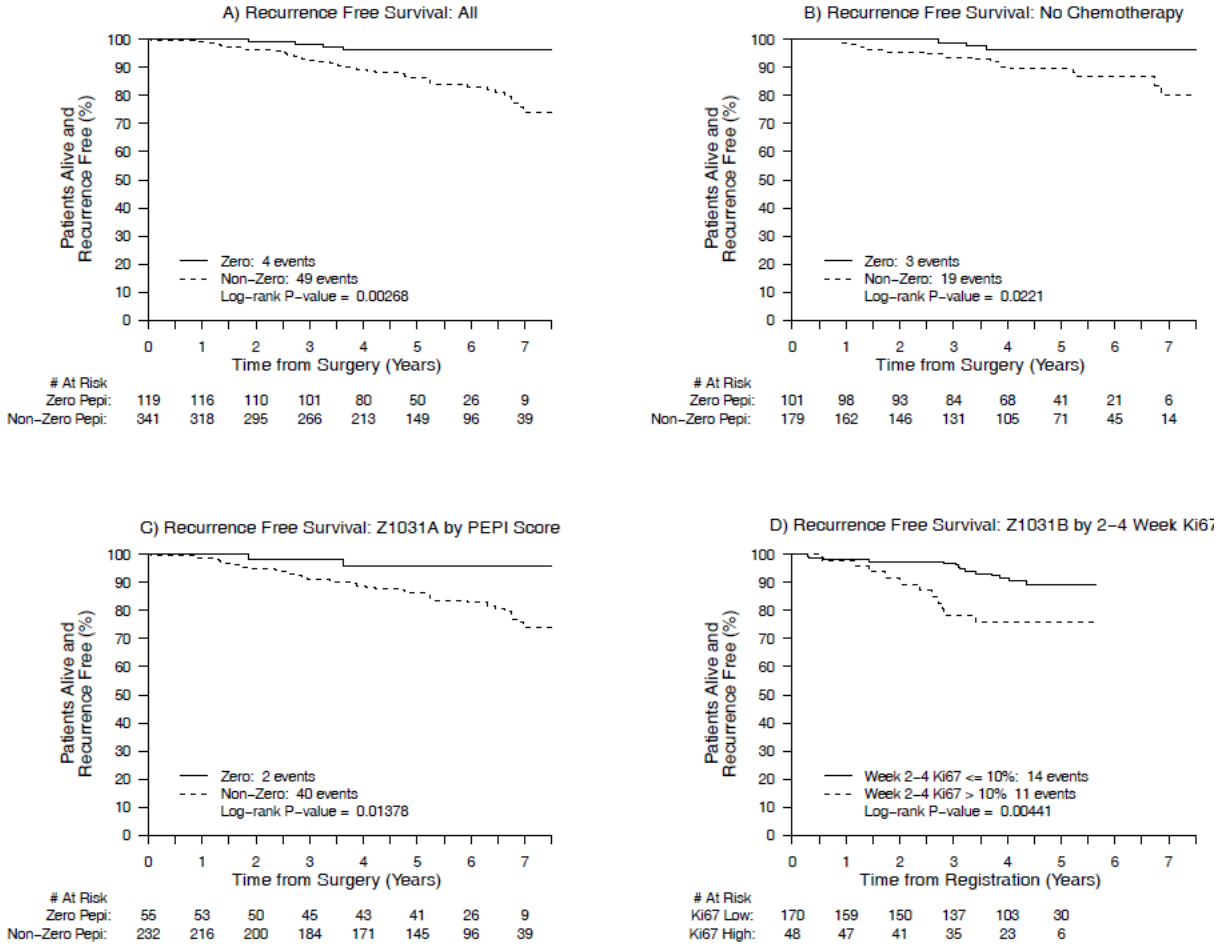


Figure 3. Kaplan Meier Analysis by PEPI=0 (pathological stage 1 or 2A, surgical specimen Ki67<2.7% and ER Allred score >2) versus PEPI>0 in all patients (panel A) and for patents who did not receive adjuvant chemotherapy (panel B) and for patients in the Z1031A cohort alone (panel C). Outcomes for patients according to the 10% Ki67 cut-point on Z1031B are displayed in panel D.

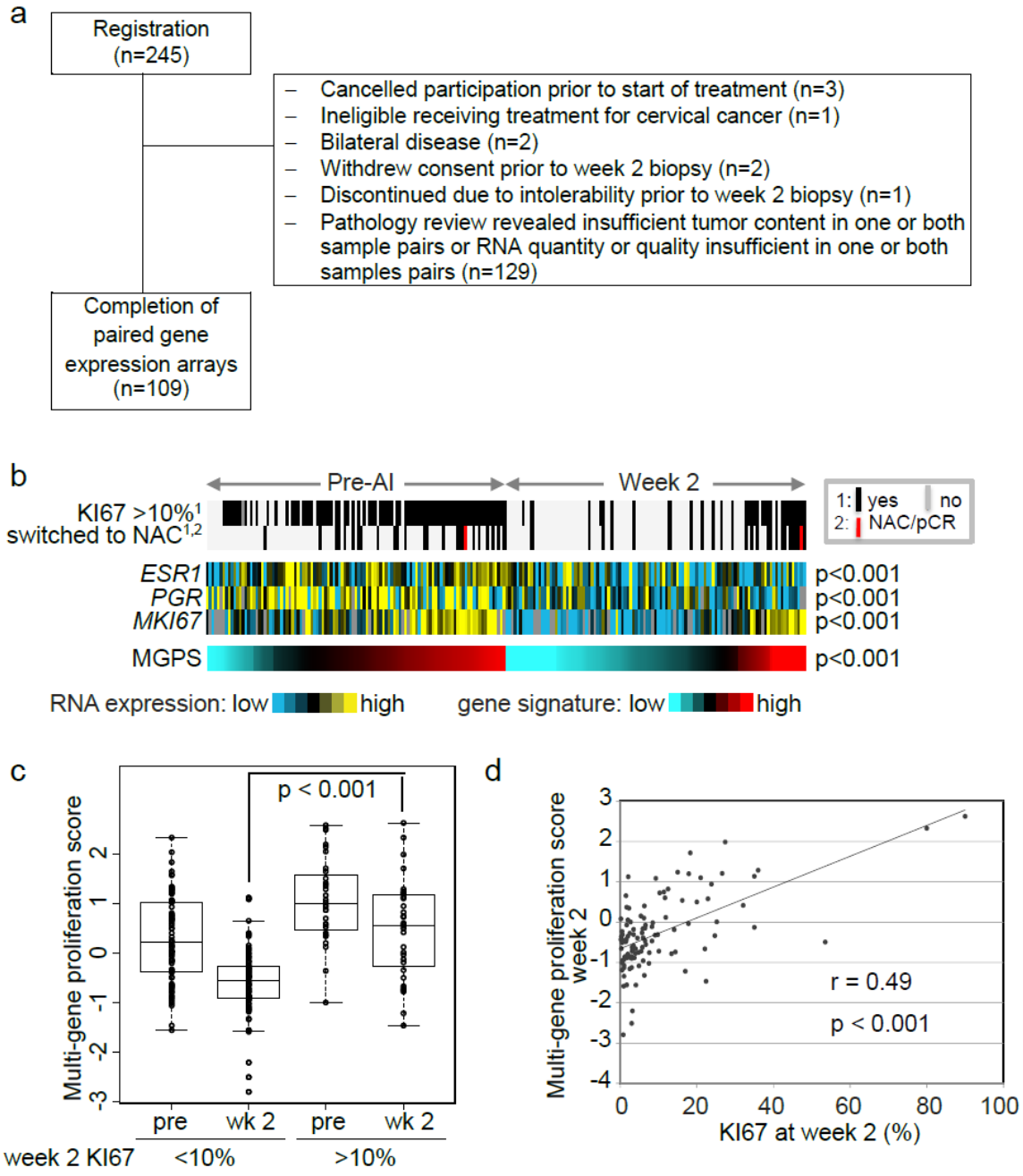


Figure 4. Gene expression based analysis of proliferation in 109 baseline ("BL") and 2 week paired samples ("2wks", including a small number of patients the where the data was derived from a 4 week sample) from the Z1031B cohort only (Panel A for REMARK diagram). Panel B: The effect of AI treatment on mRNA levels for ER, PgR and Ki67 as a heat map showing marked suppression with treatment. The grey and black bars indicate which sample is associated with Ki67 values above or below 10% and which patients received chemotherapy. The red bar indicates the single case captured in this analysis that experienced a pCR to chemotherapy. The lowest bar provides a heat map from the multi-gene proliferation score (MGPS - cell cycle) also showing marked suppression of treatment, but also identifying cases that have presently high levels of gene expression from cell cycle related genes that overlap with cases with Ki67 levels of >10% and received chemotherapy. Panel C: Box plots comparing the MGPS scores at baseline and two weeks in samples associated with 2 week Ki67<10% or >10% showing higher scores at both baseline and at 2 weeks for patients with Ki67 scores >10% (Wilcoxon signed rank test P=<0.001 for both comparisons). Panel D: Correlation between Ki67 values and MGPS values at 2 weeks with the Pearson correlation coefficient.

Table Legends

Table 1. Patient and disease characteristics for the Z1031B cohort sorted by early on treatment Ki67 categories. IQR is interquartile range

	week2 ki67 ≤10% (n=165)	week2 ki67 >10% (n=49)	week 2 biopsy no invasive disease present (n=22)
median age (IQR)	65 (58-72)	60 (55-64)	66 (58-72)
histology			
ductal	114 (69.1%)	40 (81.6%)	16 (72.7%)
lobular	34 (20.6%)	7 (14.3%)	4 (18.2%)
mixed ductal/lobular	10 (6.1%)	2 (4.1%)	0
other	7 (4.2%)	0	2 (9.1%)
grade			
1	54 (32.7%)	13 (26.5%)	8 (36.4%)
2	96 (58.2%)	22 (44.9%)	12 (54.5%)
3	15 (9.1%)	14 (28.6%)	2 (9.1%)
Her 2 positive	3 (1.8%)	2 (4.1%)	1 (4.5%)
cTstage			
T2	131 (79.4%)	32 (65.3%)	19 (86.4%)
T3	27 (16.4%)	16 (32.6%)	2 (9.1%)
T4A-C	7 (4.2%)	1 (2.1%)	1 (4.5%)
cNstage			
N0	113 (68.5%)	27 (55.1%)	21(95.5%)
N1	46 (27.9%)	19 (38.8%)	1 (4.5%)
N2	4 (2.4%)	3 (6.1%)	0
N3	1 (0.6%)	0	0
unknown	1 (0.6%)	0	0
Neoadjuvant endocrine therapy			
anastrozole	72 (43.6%)	16 (32.7%)	2 (9.1%)
exemestane	21 (12.7%)	10 (20.4%)	10 (45.4%)
letrozole	72 (43.6%)	23 (46.9%)	10 (45.4%)
week 2 ki67 value			
0-2.5%	89 (53.9%)	0	
2.6-5.0%	44 (26.7%)	0	
5.1-7.5%	20 (12.1%)	0	
7.6-10.0%	12 (7.3%)	0	
10.1-15.0%	0	19 (38.8%)	
15.1-20.0%	0	12 (24.5%)	
20.1-25.0%	0	6 (12.2%)	
25.1-50.0%	0	8 (16.3%)	
50.1-75.0%	0	1 (2.0%)	
75.1-100%	0	3 (6.1%)	

Table 2. Chemotherapy approaches and surgical outcomes among patients with a week 2 Ki67 of >10% who switched to neoadjuvant chemotherapy. SLN is sentinel lymph node, ALND is Axillary Lymph node Dissection, A is Doxorubicin, T is Taxotere; C is Cyclophosphamide, FEC is 5-fluorouracil, Epirubicin, and cyclophosphamide and FAC is 5-fluorouracil, Adriamycin, and cyclophosphamide

	n=35
Neoadjuvant chemotherapy regimen	
AC	1 (2.9%)
AC then Paclitaxel or nab-Paclitaxel	10 (28.6%)
ACT	3 (8.3%)
TC +/- trastuzumab	7 (20.0%)
FEC then docetaxel	4 (11.3%)
FEC then paclitaxel	3 (8.3%)
Paclitaxel then FAC	2 (5.7%)
Paclitaxel then FEC	2 (5.7%)
Ixabepilone/cyclophosphamide	1 (2.9%)
Taxotere + Carboplatin + trastuzumab	1 (2.9%)
Taxotere + gemcitabine + bevacizumab then AC + bevacizumab	1 (2.9%)
Extent of breast surgery	
breast conserving surgery	14 (40.0%)
modified radical mastectomy	21 (60.0%)
Extent of nodal surgery	
SLN procedure	11 (31.4%)
ALND	15 (42.9%)
SLN+ALND	9 (25.7%)
Residual disease in the breast	
none	2 (5.7%)
0.1-2.0 cm	16 (45.7%)
2.1-5.0 cm	12 (34.3%)
5.0+ cm	5 (14.2%)
Number of positive lymph nodes	
only fatty tissue identified in ALND specimen	1 (2.9%)
0	19 (55.9%)
1-3	11 (31.4%)
4-9	4 (11.3%)

Table 3. Outcomes among patients with week 2 Ki67 of ≤10% or not determined who continued on neoadjuvant aromatase inhibitor. SLN is sentinel lymph node, ALND is Axillary Lymph node Dissection and PEPI is Preoperative Endocrine Prognostic Index

	n=186
Breast surgery preformed	
none	9 (4.8%)
breast conserving surgery	115 (61.8%)
modified radical mastectomy	62 (33.3%)
Nodal surgery performed	
No nodal evaluation	10 (5.4%)
SLN procedure	114 (61.3%)
ALND	38 (20.4%)
SLN+ALND	24 (12.9%)
Residual breast disease (found on pathologic examination)	
none	3 (1.6%)
0.1-2.0 cm	73 (39.2%)
2.1-5.0 cm	85 (45.7%)
5.0+ cm	16 (8.6%)
not applicable	9 (4.8%)
Number of positive lymph nodes (found on pathologic examination)	
not examined	10 (5.4%)
0	94 (50.5%)
1-3	53 (28.5%)
4-9	18 (9.7%)
10+	11 (5.9%)
PEPI score	
0	64 (34.4%)
1-7	109 (58.6%)
non-zero	2 (1.1%)
not determinable	11 (5.9%)

Artigo 3

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Cost-effectiveness analysis of locally advanced estrogen receptor-positive, HER-2 negative breast cancer care using a tailored treatment approach.

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Running head: Cost-effectiveness analysis of tailored breast cancer treatment

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ABSTRACT

Introduction: Breast cancer is the most common cancer in women worldwide, and 70% of breast cancer deaths occur in women from low-income and middle-income countries. Neoadjuvant endocrine therapy (NET) is an attractive alternative to Neoadjuvant chemotherapy for Hormone Receptor-positive tumors.

Methods: Using the software TreeAge Pro 2017, we built a decision analysis model of breast cancer treatment to compare a NET schema, with response based in the evaluation of Ki-67, against the adjuvant chemotherapy standard-of-care as two competing approaches to breast cancer management. Our objective is to determine whether tailoring chemotherapy treatment based on response to neoadjuvant endocrine therapy is a cost-effective approach.

Results: Our model shows that, given certain assumptions, the standard-of-care strategy is dominated by the NET schema with incremental cost savings of R$32009.36 per patient for the NET strategy compared to the standard of care strategy. Cost-effectiveness of the neoadjuvant endocrine treatment strategy was R$2612.63 and R$4369.11 for the standard-of-care. Considering the standard willingness-to-pay of R$50000.00, the standard-of-care strategy would only be more cost-effective in the scenario of a Ki-67 test that misclassifies patients more than 15.4% of the time.

Conclusion: The use of response to neoadjuvant endocrine treatment based on Ki-67 analysis as a way to tailor locally advanced breast cancer treatment is a cost-saving strategy in the presence of robust biomarkers.

INTRODUCTION

Breast cancer (BC) is the most common cancer in women worldwide, and 70% of breast cancer deaths occur in women from low-income and middle-income countries.[1] Survival at five years varies from around 80% in high-income countries to 60% in middle-income countries and 40% in low-income countries. [2]

Hormone receptor positive (HR+) tumors represent the most common form of breast cancer and account for most of the deaths from this disease. Modern treatment strategies are tailored to molecular subtypes allowing a more individualized approach to therapy. Endocrine therapy is the mainstay of treatment for patients with HR+ breast cancer.

In low to middle income countries, most cases are diagnoses at later stages of disease, being already locally advanced. Locally advanced breast cancer treatment involves three main therapeutic modalities: surgery, systemic therapy, and radiation therapy. Adjuvant systemic therapies comprise the administration of drugs to treat cancer cells that cannot be addressed with surgery. These treatments include chemotherapy, hormonal therapy, immunotherapy, and biologicals such as monoclonal antibodies. Traditionally, adjuvant systemic therapy has been administered after surgery. Pivotal trials have demonstrated that neoadjuvant (preoperative) chemotherapy (NCT) is safe and equivalent to adjuvant chemotherapy regarding risk of recurrence and overall survivall[3,4]. Additionally, neoadjuvant chemotherapy induces tumor down-staging and increases rates of breast-conserving surgery (BCS)[5]. Response and benefit to NCT vary according to HR expression, with lower responses in luminal tumors compared to HR-negative and HER2 positive tumors. Neoadjuvant endocrine therapy (NET) is an attractive alternative to NCT for HR-positive tumors, since it is simple to deliver and a well-tolerated targeted therapy[6]. The potential of NET is being widely explored, not simply to allow less extensive surgery but also to promote personalized medicine. Neoadjuvant trial outcomes have successfully predicted outcomes both in adjuvant trials[7] and for the individual patient.[8] At the present time, ideal candidates for NET include postmenopausal patients with ER-enriched stage II and III breast cancer. In this setting, clinical trials demonstrated that aromatase inhibitors (AIs) are superior to tamoxifen[9-11]. Currently there are three available AIs (anastrozole, letrozole and exemestane) and they have equivalent effects[12]

Recently, the American College of Surgeons published the results of the Z1031 cohort B trial. In this trial, response to neoadjuvant endocrine treatment based on the Ki-67 biomarker was used to select patients that could be spared from chemotherapy[13] 236 patients were enrolled to receive neoadjuvant endocrine treatment and had a second biopsy after 2 weeks of treatment. If Ki-67 was higher than 10% after 2 weeks, that patient would be triaged to chemotherapy. If Ki-67 was lower than 10%, then the patient would remain on neoadjuvant endocrine treatment for 16-18 weeks. The primary endpoint of that trial was pathological complete response among women who after 2 weeks of neoadjuvant endocrine treatment had a Ki-67 level higher than 10%. Secondary endpoints of this trial included the rate of Preoperative Endocrine Prognostic Index (PEPI) score zero and the acceptance of a recommendation of treatment without adjuvant chemotherapy in this latter group. With that in mind, we built a decision analysis model of breast cancer treatment to compare the Z1031 cohort B schema against the adjuvant chemotherapy standard-of-care as two competing approaches to breast cancer management. Our objective is to determine whether tailoring chemotherapy treatment based on response to neoadjuvant endocrine therapy is a cost-effective approach in the Brazilian public health system (SUS).

METHODS

Approach

The methods, patient characteristics (can be found in Appendix, since those are the data used for the Adjuvant! Online survival estimates) and trial results of the ACOSOG Z1031 have been previously reported[12] We developed a mathematical model to estimate the economic outcomes of the strategy of triaging patients to chemotherapy based on response to neoadjuvant endocrine treatment, in women with ER+, HER2 negative, stage 2 or 3 BC. The last step of the model included a Markov chain in which patients would navigate between 4 health states, follow-up, local relapse, metastatic disease and death. Data from the ACOSOG Z1031 [12], NSABP-B18 [14] and Adjuvant! Online [15] were used to estimate patient survival. Adjuvant! Online is a web-based application designed to provide 10-year survival probability of patients with BC according to different treatment decisions. Literature sources were used to identify utilities (quality-of-life)[16]. Cost of interventions, such as cost of drugs, cost of surgery, cost of radiation therapy and other costs, were identified in the Brazilian Health Ministry database[17].

Study design and model structure

We constructed a decision-analytic model, using TreeAge Pro™ software (TreeAge Software, Inc, MA, USA), to consider two modalities of treatment described below. In the first modality, patients start neoadjuvant endocrine treatment with an aromatase inhibitor (AI) and the second modality reflects standard-of-care. Both treatment modalities included breast surgery, radiation therapy and adjuvant endocrine treatment for 5 years (figure 1). Lumpectomy (BCS) or mastectomy were the surgical modalities accepted, according to the tumor size at the moment of surgery. Each of the intervention arms ended in a Markov process. In these processes the subjects would navigate between 4 different health states: dead, follow-up without disease, local relapse or metastasis. The model kept running until all the subjects were dead, either from the cancer itself or from other causes. The inputs for the Markov process were derived from Adjuvant! Online. [15] We evaluated healthcare costs from a provider's perspective, which is the Brazilian Public Health System, denominated "Sistema Único de Saúde – SUS". The main assumption on this model is that the Ki-67 biopsy is a reliable method to triage patients to chemotherapy, not allowing the misclassification of patients. Also, we used the assumption that both treatment strategies have similar clinical outcomes, based on the results of NSABP B-18[14] and ACOSOG Z1031B[13].

Neoadjuvant endocrine treatment group:

Patients received an AI for 4 weeks. A second biopsy was then performed to determine the Ki-67 score. Patients were stratified according to Ki-67 score into two groups. Patients with Ki-67 < 10% continue with the AI treatment for 16-18 weeks followed by surgical treatment. After surgery, this group of patients can either remain with only AI with no recommendation for adjuvant chemotherapy, if the PEPI score is equal to zero, or get adjuvant chemotherapy, if the PEPI score is $\geq$ 1 (A description of the PEPI score can be found in the appendix). Patients with Ki-67>10% are immediately triaged to neoadjuvant chemotherapy for 4-8 cycles, according to the physician's discretion, followed by surgical treatment. The inputs for Ki-67 (<10% vs. $\geq$10%), PEPI score (zero vs. $\geq$1) and type of surgery were derived from the literature and can be found in table 1. All patients received radiation therapy due to locally advanced disease, according to international guidelines.

Standard-of-care group

Patients received mastectomy as surgical treatment followed by adjuvant chemotherapy (4-8 cycles, according to the physician's discretion) and adjuvant endocrine

treatment with an AI for 5 years. All patients received radiation therapy due to locally advanced disease, according to international guidelines.

Patient population

The patient population consisted of post-menopausal women with ER+, HER2 negative, stage 2 or 3 breast cancer. The patient population in the model had the same age distribution and tumor characteristics as described in the ACOSOG Z1031 trial. [12]

Disease-free and overall survival inputs

The disease-free survival and the overall survival inputs were all derived from calculations on the Adjuvant! Online platform. Adjuvant! Online allows you to calculate those values based on patient's characteristics, tumor characteristics and also treatment regimens adopted. The "model patient" entered in the Adjuvant! Online platform had average co-morbidities based on age, ER status positive, tumor grade=2, tumor size= 3.1-5, 1-3 positive nodes, had AI for 5 years as hormonal therapy and "CAx4 then Tx4" as chemotherapy regimen; age varied according to the patient characteristics of the ACOSOG Z1031 trial (see appendix). By varying the age of the subject, between 50 and 80 years of age, two tables were constructed (see appendix), one for mortality and one for relapse-free survival. The Markov model used these values to determine mortality and disease progression according to the patient's age when entering the model.

Quality-of-life

As we don't have utility values based on Brazilian cohorts, utility values determined by a Canadian study [16] were applied to the different health states in our Markov model (table 2).

Cost inputs

Cost inputs for the whole model were obtained in the Brazilian Health Ministry webpage[17]. The detailed costs according to treatment modalities that were included in the model are shown in table 3. The values we used as inputs to our model are based on charges from the hospital to SUS, and are filed by the doctors after each treatment modality. The values are standard to any hospital in the country that works under the SUS organization. Costs for office visits to the oncologist, breast surgeon and radiation oncologists are also charged.

Study outcomes

Our primary endpoint was to evaluate whether the Z1031B strategy was cost-effective when compared to the standard-of-care in Brazil. We also included a sensitivity analysis to assess the impact of the failure of the Ki-67 test on the cost-effectiveness analysis.

RESULTS

Our model shows that, given the stated assumptions, the standard-of-care strategy is dominated in this setting, as its cost is higher for the same effectiveness. The neoadjuvant endocrine treatment strategy showed a slightly higher effectiveness, of 18.30 against 18.27 from the standard-of-treatment strategy. The cost of the neoadjuvant endocrine treatment

strategy was R$ 47799.89 per patient. The incremental cost savings were R$32009.36 per patient for the neoadjuvant endocrine treatment strategy compared to the standard of care strategy. Cost-effectiveness of the neoadjuvant endocrine treatment strategy was R$2612.63 and R$4369.11 for the standard-of-care. The main results are presented in table 4. The parameters that had the largest impact on cost were neoadjuvant chemotherapy and adjuvant chemotherapy.

Our model suggests that a failure of 15% on the Ki-67 test would result in an Incremental Cost-Effectiveness Ratio (ICER) of R$51324.21 for the standard of care strategy. The sensitivity analysis on the impact of failure of the Ki-67 test is shown in figure 2. Considering the standard willingness-to-pay of R$50000.00, the standard of care strategy would be more cost-effective in the scenario of a Ki-67 test that misclassifies patients more than 15.4% of the time.

DISCUSSION

Considering our aim to evaluate the tailored approach to cancer treatment, this modeled analysis indicates that neoadjuvant endocrine treatment is a more cost-effective strategy than the standard-of-care. The case analysis for a stage 2 or 3, ER+, Her2 negative, BC patient demonstrated that the additional biopsy for Ki-67 and tailored treatment decision could result in a significant cost saving of R$32009.36 per patient. In a country such as Brazil, with an estimate of 57960 new breast cancer cases in 2016, where 64% of the cases are stage 2 or 3, that cost saving would have a tremendous impact on the public health system.

The Brazilian public health system face many challenges caring for patients with breast cancer: inadequate funding; inequitable distribution of resources and services; lack of adequate transportation strategies for patients outside major urban areas; inadequate distribution of health-care personnel and equipment; and lack of adequate care for many population based on socioeconomic, geographic, ethnic and other factors.[18]

Chemotherapy has been the mainstay modality in the neoadjuvant treatment of breast cancer since the landmark NASBP B-18 trial[4]. Since then, characterization of breast cancer subtypes has directed us to a more rational treatment approach to the disease[19]. The chemotherapy strategy is particularly adequate for triple negative breast cancers and HER2 positive disease (in combination with anti-HER2 agents), with a consistent high pathologic complete response. At the same time, the added benefit of chemotherapy for the larger and heterogeneous HR+ subgroup has been challenged.[20]

NET is a rational approach that provides an outstanding opportunity to further individualize treatment selection. NET has a favorable toxicity profile and is associated with benefits such as having low cost and being more easily available even for cancer care professionals outside major urban areas or tertiary centers.[6] These factors are particularly relevant as 70% of breast cancer deaths occur in women from low-income and middle-income countries. However, it has been timidly evaluated in clinical trials and even more so implemented in clinical practice, even in developed countries. According to the National Cancer Data Base in the U.S. only 3% of the eligible patients receive this therapy.[21] There are no specific data regarding the use of NET in current clinical practice in Brazil. Nevertheless, we expect that the number is even lower than in the US for a variety of reasons like limited knowledge of NET even between medical oncologists and breast surgeons, deficiencies regarding access to pathology and radiology tests as well as reimbursement issues.

The main limitation of this model is that it is dependent on the assumption that the Ki-67 test is correct 100% of the time. It is quite obvious that no test can provide that accuracy and efficiency. As the test becomes inaccurate, the number of patients not being triaged to chemotherapy rises. This way, patients that would benefit from chemotherapy are erroneously spared and will have an increased chance of relapse and death due to cancer. The likelihood of death due to cancer and relapse in the absence of adequate treatment can be calculated via Adjuvant! Online.

Ki-67 is one of the most widely used proliferation markers in breast cancer. Although the existing guidelines of the American Society of Clinical Oncology do not include it in the list of required biomarkers [22], recent papers established an increasing importance in its role. During St. Gallen International Expert Consensus meeting in 2013, the panel already advised for the use of immunohistochemical definition of estrogen and progesterone receptor, HER-2 oncogene and Ki-67 as means of defining tumor subtypes. The panel also addressed the importance of Ki-67 as fundamental for the distinction between Luminal A and Luminal B tumors[23]. The International Ki67 in Breast Cancer Working Group also acknowledges the importance of Ki-67 as a pharmacodynamic intermediate endpoint and an eligibility criterion for neoadjuvant trials. [24] A recent paper by Luporsi et al[25] identified 17 studies that analyzed samples from patients that had been included in RCTs (Randomized Controlled Trials) and with centralized slide review in neoadjuvant and adjuvant setting and it attributed a level of evidence I-B validating the use of Ki-67 as a prognostic factor for disease-free survival in patients receiving adjuvant therapy. However, Colozza[26] et al. states clearly the need for Ki-67 pathological assessment standardization before this biomarker can be introduced as routine practice or be part of clinical decision scores.

Despite the caveats associated with Ki67 evaluation, our analysis shows that a tailored approach based on NET for locally advanced ER+ breast cancer is associated with lower cost and slightly higher effectiveness compared to standard-of-care. In the setting of low to middle-income countries, additional benefits of NET include favorable toxicity profile, oral administration and treatment availability even outside reference centers and major urban areas.

CONCLUSION

The use of response to neoadjuvant endocrine treatment based on Ki-67 analysis as a way to tailor locally advanced breast cancer treatment is a cost-effective strategy in the presence of robust biomarkers. If incorporated in the Brazilian health system, this strategy would benefit both patients and the system itself. Patients would receive earlier access to systemic treatment, which could impact their overall survival, and the public health system managers would have extra resources to invest in other areas, such as cancer screening and early-diagnosis programs.

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Figure Legends

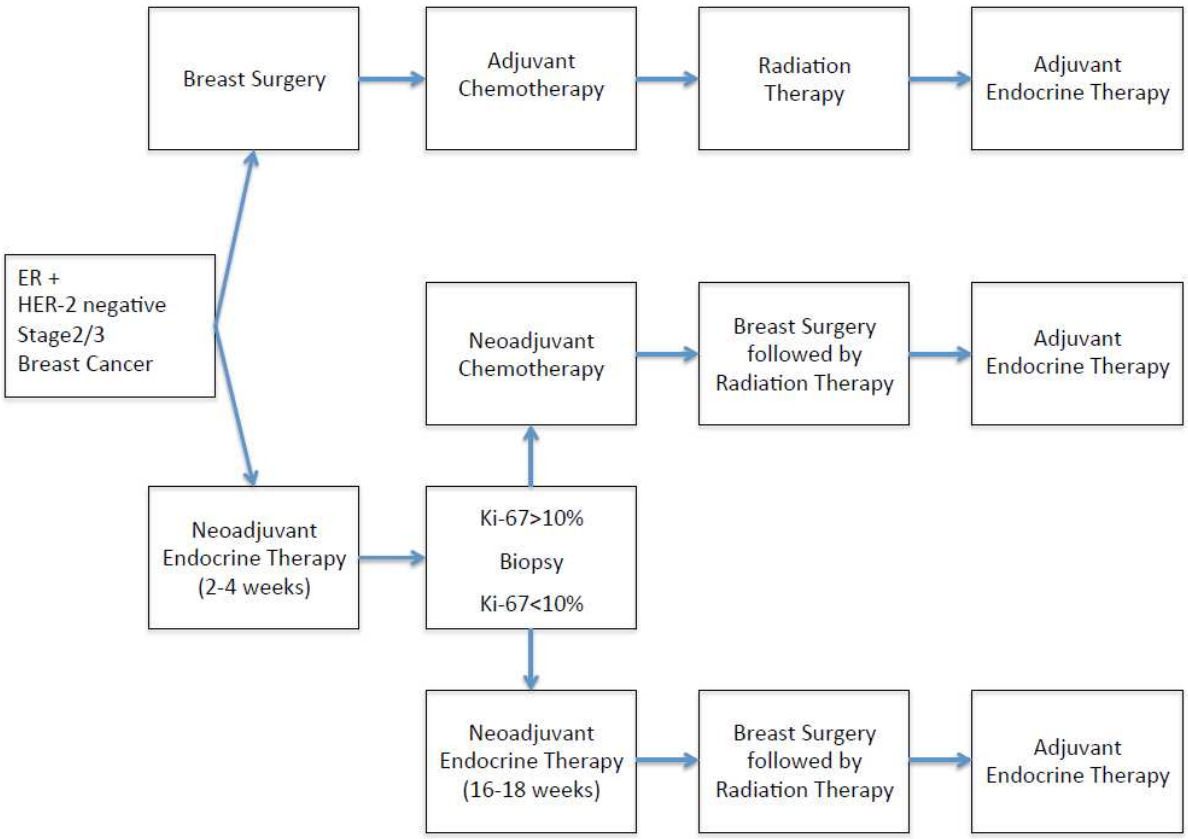

Figure 1: Model Schema for the design of the decision analysis model

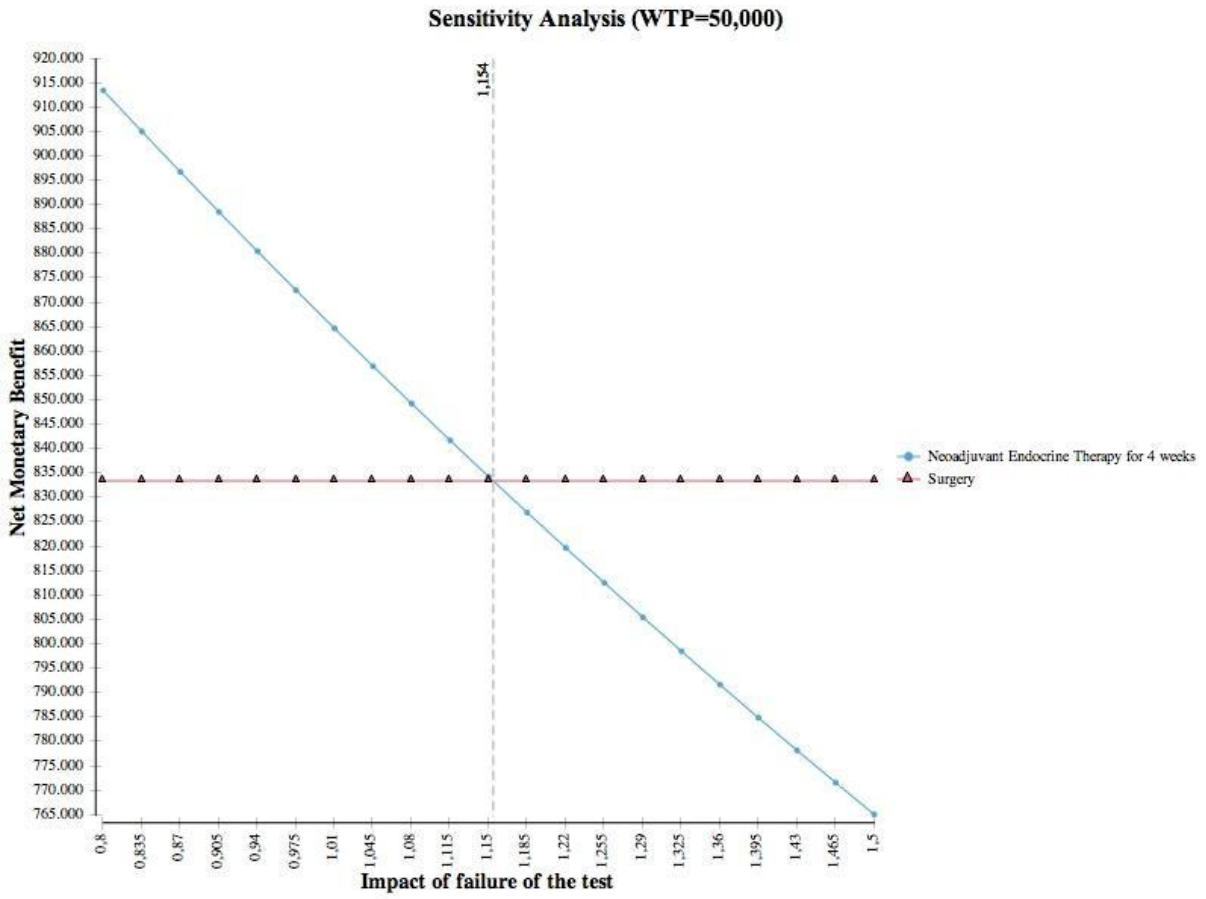

Figure 2: As the probability of the failure of the test increases, the standard-of-care approach (surgery) becomes more cost-effective. Beyond the point where the probability of the test failing is 15.4%, surgery is not dominated anymore and the ICER is below the Willingness-to-pay (WTP) of R$50000.00

Table 1. Probability inputs used for building the model.

Parameter	Probability	
Ki-67<10%	0.79	ACOSOG-Z1031[12]
Ki-67>10%	0.21	ACOSOG-Z1031[12]
PEPI score=0	0.37	ACOSOG-Z1031[12]
PEPI score>0	0.63	ACOSOG-Z1031[12]
BCS (HT)	0.51	ACOSOG-Z1031[12]
Mastectomy (HT)	0.49	ACOSOG-Z1031[12]
BCS (Chemotherapy)	0.637	NSABP-B18[4]
Mastectomy (Chemo)	0.363	NSABP-B18[4]
Progression to Metastasis	Variable	Adjuvant! Online[15]
Death of other causes	Variable	Adjuvant! Online[15]
Death of Cancer	Variable	Adjuvant! Online[15]

Table 2. Utility inputs used for building the model. [16]

Health state	Utility
Disease-free	0.9
Local relapse	0.7
Metastasis	0.6
Death	0.0

Table 3. Cost inputs used for building the model in Brazilian currency (R$)[17]

Treatment	Cost (R$)
Biopsy	182.18
Lumpectomy	1913.83
Mastectomy	2462.85
Local relapse resection	2045.07
Neoadjuvant Endocrine Treatment	478.50
Neoadjuvant Chemotherapy	8400.00
Radiotherapy following Lumpectomy	3776.00
Radiotherapy following Mastectomy	3776.00
Adjuvant Endocrine Treatment	4785.00
Adjuvant Chemotherapy	6400.00
Follow-up of metastatic patient	412.48
Palliative chemotherapy	10200.00

Table 4. Cost-effectiveness analysis main results

Treatment	Cost (R$)	Incremental Cost (R$)	Effectiveness	Incremental Effectiveness	Cost/ Effectiveness
Surgery	79809.24	32009.36	18.27	-0.03	4269.11
Neoadjuvant Endocrine Therapy	47799.89	-	18.30	-	2612.63

Appendix

Mortality table, derived from Adjuvant! Online

Age	Alive	Dead of other causes	Dead of cancer
50	78.1	4	17.9
51	77.8	4.4	17.8
52	77.3	4.8	17.9
53	76.9	5.3	17.8
54	76.4	5.8	17.8
55	75.9	6.3	17.8
56	75.2	6.9	17.9
57	74.7	7.5	17.8
58	74.1	8.2	17.7
59	73.3	8.9	17.8
60	71.4	9.6	19
61	70.6	10.4	19
62	69.8	11.3	18.9
63	68.9	12.2	18.9
64	67.9	13.2	18.9
65	66.8	14.3	18.9
66	65.7	15.5	18.8
67	64.6	16.6	18.8
68	63.1	18.1	18.8
69	61.6	19.7	18.7
70	60	21.3	18.7
71	58.3	23	18.7
72	56.6	24.9	18.5
73	54.6	27	18.4
74	52.5	29.2	18.3
75	50	31.6	18.4
76	47.5	34.3	18.2
77	44.6	37.4	18
78	41.7	40.4	17.9
79	38.4	43.8	17.8
80	35	47.4	17.6

Relapse table, derived from Adjuvant! Online

Age	Alive	Dead of other causes	Relapse
50	75.7	3.4	20.9
51	75.2	3.8	21
52	74.8	4.1	21.1
53	74.4	4.5	21.1
54	73.9	4.9	21.2
55	73.4	5.4	21.2
56	72.8	5.9	21.3
57	72.3	6.4	21.3
58	71.6	7	21.4
59	70.9	7.6	21.5
60	67.9	8.3	23.8
61	67.1	9	23.9
62	66.3	9.7	24
63	65.4	10.5	24.1
64	64.5	11.4	24.1
65	63.5	12.3	24.2
66	62.4	13.3	24.3
67	61.4	14.3	24.3
68	59.9	15.6	24.5
69	58.6	16.9	24.5
70	57.1	18.3	24.6
71	55.4	19.8	24.8
72	53.7	21.5	24.8
73	51.8	23.2	25
74	49.8	25.2	25
75	47.5	27.3	25.2
76	45	29.6	25.4
77	42.2	32.3	25.5
78	39.5	34.8	25.7
79	36.4	37.8	25.8
80	33.2	40.9	25.9

Patient characteristics of Z1031

Baseline Patient Characteristics by Assigned Treatment Arm

Characteristic	Anastrozole (n = 123; %)
Age, years	
Median	65
Range	51-87
Race	
White	82.9
Black/African-American	13.0
Unknown	4.1
Postmenopausal status	
Bilateral oophorectomy	23.6
FSH and estradiol in postmenopausal range	9.8
Amenorrhea for > 1 year	66.7
ECOG performance status	
1	74.8
2	19.5
3	5.7
Clinical T stage	
T2	76.4
T3	19.5
T4a-c	4.1
Clinical N stage	
N0	74.0

Characteristic	Anastrozole (n = 123; %)
N1	23.6
N2	2.4
N3	—
Maximum tumor dimensions by caliper exam, cm	
2.0-2.9	24.4
3.0-3.9	21.1
4.0-4.9	17.9
≥ 5.0	35.8
Unknown	0.8
Histologic grade	
1	24.4
2	59.4
3	15.4
Unknown	0.8
Histologic type	
Ductal	77.2
Lobular (≥ 90% of specimen)	17.1
Other	5.7
Allred score (local laboratory results)	
6	21.1
7	18.7
8	60.2
HER2/neu status (local laboratory results)	

Characteristic	Anastrozole (n = 123; %)
Positive	9.8
Negative	87.8
Not done	2.4
Surgical status at presentation	
Marginal lumpectomy candidate	57.7
Modified radical mastectomy candidate	41.5
Inoperable	0.8

Abbreviations: FSH, follicle-stimulating hormone; ECOG, Eastern Cooperative Oncology Group; HER2, human epidermal growth factor receptor 2.

PEPI score

Neoadjuvant endocrine therapy has been widely adopted as a practical means to improve surgical outcomes for postmenopausal women with ER+ stage 2 and 3 breast cancer, but little was known about how the post–neoadjuvant endocrine therapy pathological stage and biomarker status could be used to make decisions regarding other adjuvant treatments. To address this question, Ellis et al integrated information on standard pathological staging parameters after neoadjuvant endocrine therapy with measurements of ER status and levels of the Ki67 proliferation antigen in the surgical specimen to create the PEPI score that weights these factors according the magnitude of the HR. Of particular note, patients with low pathological stage (stage 1 or 0) and a favorable biomarker profile (PEPI score 0) at surgery had such a low rate of relapse that further adjuvant systemic therapy beyond continuation of an endocrine agent appears unnecessary. In striking contrast, patients with high pathological stage disease at surgery and a poor biomarker profile (PEPI group 3) had a statistically significant higher risk of early relapse, more typical of ER _ disease, and therefore should be offered all appropriate adjuvant treatments available.

Reference

Outcome Prediction for Estrogen Receptor – Positive Breast Cancer Based on Post-neoadjuvant Endocrine Therapy Tumor Characteristics

Authors: Matthew J. Ellis , Yu Tao , Jingqin Luo , Roger A'Hern , Dean B. Evans , Ajay S. Bhatnagar ,Hilary A. Chaudri Ross , Alexander von Kameke , William R. Miller , Ian Smith ,

Wolfgang Eiermann , Mitch Dowsett

V. DISCUSSÃO

O trabalho apresentado nesta tese demonstrou que é possível padronizar o biomarcador Ki-67 e que o seu uso tem implicações muito importantes tanto do ponto de vista clínico como do ponto de vista econômico no manejo de pacientes com câncer de mama do subtipo molecular luminal. Este estudo iniciou-se pelo desenvolvimento de um método padronizado de avaliação do Ki-67. Essa metodologia foi em seguida validada em outra coorte comprovando a associação do nosso método com desfechos clínicos relevantes como a sobrevida global, sobrevida livre de doença e uma estimativa do benefício do uso de quimioterapia em pacientes de acordo com o valor do Ki-67. Além disso, avaliamos que a implementação do uso do Ki-67 como determinante da individualização de tratamento em pacientes do SUS seria capaz de aperfeiçoar o uso de recursos financeiros públicos.

Nós desenvolvemos um método de avaliação de Ki-67 eficiente e reprodutível que foi aprovado pelo Programa de Avaliação de Estudos Clínicos (Clinical Trials Evaluation Program - CTEP) para estudos patrocinados pelo National Cancer Institute dos EUA que avaliem endocrinoterapia neoadjuvante. A combinação de análise de imagens por software com triagem para contagem manual, quando necessário, respeita o fato de que o software de análise de imagens nem sempre diferencia certos tipos de células normais de células neoplásicas. Essa estratégia também enfatiza o papel fundamental do patologista na avaliação das imagens digitalizadas para determinar o método de contagem de Ki-67 mais apropriado (software ou contagem visual) quando a histologia é complexa. O fluxograma das amostras ilustra como, enquanto diferentes patologistas podem ter diferentes interpretações da necessidade de contagem visual, essas diferenças não afetam o poder de predizer os desfechos clínicos. A taxa de triagem para contagem visual foi, em media 16% no nosso primeiro estudo[45] e cerca de 6% no estudo Z1031[33], demonstrando que o software de análise de imagens pode ser usado na maioria dos casos, reduzindo consideravelmente a necessidade de realizar a trabalhosa e demorada contagem visual para apenas um número pequeno de casos. Alguns blocos de tumores dos estudos POL[25] e P024[27] infelizmente já haviam sido exauridos por análises prévias e, por tal motivo, apresentamos resultados em

tamanhos amostrais reduzidos. Portanto, a validação dessa metodologia nesses casos, apesar de promissora, não produz evidências suficientes para introdução imediata na prática clínica diária. Para obter tais evidências, será necessária a execução de um estudo prospectivo desenhado especificamente para demonstrar que pacientes que obtenham um score PEPI-0, através da nossa metodologia de avaliação de Ki-67, podem ser tratados de maneira segura sem a necessidade de quimioterapia.

Quando o Food and Drug Administration (FDA) avaliou o uso de Ki-67 no cenário de endocrinoterapia neoadjuvante, a entidade concluiu que o algoritmo proposto não acrescenta risco significativo uma vez que reduz o risco de sub-tratamento. Chegou-se a essa conclusão baseando-se na análise do uso de quimioterapia de acordo com o score PEPI. Ficou demonstrado que oncologistas clínicos que se baseiam apenas no grau histológico após endocrinoterapia neoadjuvante acabam por indicar quimioterapia adjuvante a um número menor de pacientes. A análise combinada do P024, IMPACT e POL demonstrou que apenas 8% dos pacientes com estádio patológico 1 ou 2A receberam quimioterapia adjuvante (Tabela S2 do artigo 1). Dessa forma, o FDA considerou que o conhecimento do valor do Ki-67 em uma amostra de tumor reduziria o risco de sub-tratamento para pacientes com estádio patológico inicial (1 ou 2A) porém com características biológicas agressivas, como um Ki-67 alto em vigência de tratamento.

Um aspecto não abordado em nossa estratégia de avaliação de Ki-67 se refere ao fato de existirem casos em que a coloração pela imunoistoquímica não é uniforme – tanto a contagem visual como o software de avaliação de imagens requerem campos aleatórios. Em nossa análise, consideramos uma abordagem que não leva em consideração a heterogeneidade dos tecidos, a mesma utilizada em estudos de genômica. Apesar de reconhecermos que a análise de heterogeneidade, ou “hot-spots”, deva ser investigada, esse é um problema complexo para o qual será necessário o desenvolvimento de uma definição de “hot-spot” associada a desfechos clínicos primeiramente num conjunto de amostras de treinamento e que seja confirmada num conjunto de amostras de validação, diferente do primeiro.

Outro tópico controverso é o ponto de corte do Ki-67 como substituto para a diferenciação entre o subtipo molecular Luminal A do subtipo Luminal B em

pacientes com câncer de mama. Em nossa presente análise, um ponto de corte de 10% apresenta melhores características em uma curva ROC, enquanto uma publicação mais recente, em uma amostra diferente de pacientes, utilizando metodologia similar, sugeriu 14% como ponto de corte ideal [46], sugerindo dessa forma a possibilidade um intervalo estreito de valores para essa finalidade. Do ponto de vista do nosso estudo, o ponto de corte de 10% foi mais conservador e serve ao propósito da identificação precoce de pacientes com tumores do subtipo luminal B com características de resistência ao tratamento endócrino. A evolução rápida da doença para pacientes com Ki-67>10%, apesar da terapia com inibidores de aromatase, deixa clara a importância de desenvolvermos uma estratégia de tratamento robusta para essa população de alto risco.

Esses resultados são fruto de 4 anos de estudos na padronização do biomarcador Ki-67 e permitiram que obtivéssemos junto ao FDA a autorização para aplicação do método no estudo ALTERNATE [35].

O nosso segundo artigo, publicado recentemente[34], tratou de validar em uma casuística maior e com longo tempo de seguimento a técnica de aferição do biomarcador Ki-67 desenvolvida em nosso primeiro artigo. Nesse estudo, ficou demonstrado que a determinação do score PEPI através da análise do Ki-67, é capaz de identificar um grupo de pacientes com risco de recidiva muito baixo, de 3%, em 5 anos e que, portanto, não terá benefício em receber quimioterapia adjuvante. As limitações do uso do Ki-67 foram discutidas de maneira extensa anteriormente nessa tese. Afim de contornar tais críticas constantes na literatura com relação ao uso do Ki-67, utilizamos um score de proliferação multigênico que demonstrou a elevada expressão de genes relacionados à proliferação e ao ciclo celular quando o valor de Ki-67 era superior a 10% após 2 a 4 semanas de endocrinoterapia neoadjuvante. Dessa forma, nossos dados fornecem maior evidência do valor prognóstico do biomarcador Ki-67, dentro do PEPI score, em pacientes com câncer de mama com expressão de receptores de estrógeno submetidas a endocrinoterapia neoadjuvante.

Em nosso terceiro estudo, avaliamos uma estratégia individualizada de tratamento em pacientes com câncer de mama RE+, HER-2 negativo, estádio 2 ou 3. Em nosso modelo, o uso de endocrinoterapia neoadjuvante nessas pacientes com

triagem para quimioterapia baseando-se no valor do Ki-67, mostrou-se menos dispendioso que o modelo de tratamento padrão atual no SUS, resultando em uma economia significativa de R$32009,36 por paciente. Em um país como o Brasil, com uma estimativa de 57960 novos casos de câncer de mama em 2016 segundo o Instituto Nacional do Câncer, onde 64% dos casos são diagnosticados em estádio 2 ou 3, essa economia poderia ter um significativo impacto no Sistema Único de Saúde.

Entretanto, a principal limitação desse modelo consiste na sua dependência da premissa de que a avaliação de Ki-67 é correta 100% do tempo. É bastante óbvio que nenhum teste diagnóstico é capaz disso. Ao passo que a acurácia do teste diminui, o número de pacientes que não é triado para quimioterapia aumenta. Dessa forma, pacientes que apresentariam um benefício com o uso de quimioterapia são poupados equivocadamente e apresentarão uma maior chance de recidiva e morte devido ao câncer. A chance de morte devido ao câncer e recidiva na ausência de tratamento adequado pode ser calculada através da ferramenta Adjuvant!Online[44] e essa hipótese foi testada em nosso modelo. A análise de sensibilidade do impacto do erro de avaliação do Ki-67 demonstrou que, assumindo uma “willingness-to-pay” arbitrária de R$50000,00, a estratégia de tratamento atual seria mais custo-efetiva, apenas no cenário de um teste de Ki-67 pouco confiável com erro de classificação superior a 15,4%.

Apesar de toda controvérsia na literatura sobre a reprodutibilidade do Ki-67, Luporsi et al[47] identificaram 17 estudos que analisaram amostras de pacientes incluídos em estudos clínicos randomizados (ECR), de tratamento adjuvante e neoadjuvante, com revisão central de exames anatomopatológicos. Os autores concluíram que o Ki-67 estava significantemente associado a sobrevida livre de doença em análises multivariadas de 7 ECRs e em duas meta-análises com razões de risco e riscos relativos consistentes. Ainda nesse estudo, os autores atribuem um nível de evidência I-B e validam o uso de Ki-67 como fator prognóstico para sobrevida livre de doença em pacientes recebendo terapia adjuvante.

O nosso modelo demostrou que o uso de endocrinoterapia neoadjuvante e avaliação de Ki-67 durante o tratamento é uma estratégia que pode economizar recursos na presença de biomarcadores robustos.

Apesar de existirem ainda algumas barreiras para a implementação do Ki-67 fora de estudos clínicos, o nosso estudo demonstrou que a padronização do biomarcador é possível e é capaz de economizar recursos do sistema público de saúde bem como fornecer resultados confiáveis e seguros para as pacientes.

Com base em nossos resultados, o FDA autorizou a utilização do Ki-67, com a nossa metodologia, no ALTERNATE trial[35], um grande estudo multicêntrico, prospectivo, randomizado, com o potencial de fornecer evidências definitivas para a introdução do uso do Ki-67 na prática clínica diária.

VI. CONCLUSÕES

- **Artigo 1:** Nós desenvolvemos um método de avaliação do biomarcador Ki-67 eficiente e reprodutível que foi aprovado pelo Clinical Trials Evaluation Program (CTEP) para estudos clínicos financiados pelo Instituto Nacional do Câncer dos EUA (NCI). A combinação de análise de imagem assistida por computador e triagem para análise manual, quando necessária, respeita o achado que o computador nem sempre é capaz de diferenciar certos tipos de células normais de células malignas e enfatiza o papel do patologista na revisão das decisões tomadas pelo software.

- **Artigo 2:** Nossos dados demonstram a necessidade de se avaliar o prognóstico de pacientes com câncer de mama RE+ baseando-se em características do tumor após tratamento endócrino neoadjuvante. Individualizar o tratamento baseado na falha de supressão do Ki-67 é factível. O score PEPI, baseado no valor de Ki-67 após tratamento endócrino neoadjuvante é capaz de identificar um grupo de mínimo risco de recidiva que pode ser manejado sem o uso de quimioterapia adjuvante. A metodologia de análise do Ki-67 usada nesse estudo é promissora e aguarda resultados definitivos do estudo ALTERNATE.

- **Artigo 3:** O uso do Ki-67 no SUS como método de individualização de tratamento em pacientes com câncer de mama RE+, HER-2 negativo, estádio 2 ou 3, é uma estratégia menos dispendiosa que a estratégia atualmente utilizada, resultando em uma economia de R$32009,36 por paciente.

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VIII. ANEXOS

Washington University in St. Louis

Human Research Protection Office

Barnes Jewish Hospital
St. Louis Children's Hospital
Washington University

IRB ID #: 201107392

To: Michael Naughton

From: The Washington University in St. Louis Institutional Review Board,
WUSTL DHHS Federalwide Assurance #FWA00002284
BJH DHHS Federalwide Assurance #FWA00002281
SLCH DHHS Federalwide Assurance #FWA00002282

Re: A Phase 2 Trial of 4 months preoperative Letrozole 2.5 mg daily for postmenopausal women with estrogen receptor positive and/or progesterone receptor positive T2, T3, T4a-c, N0-2, M0 breast cancer - Novel Biomarkers for Aromatase Inhibitor Therapy

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Approval Date: 01/20/15
Next IRB Approval Due Before: 01/19/16

Type of Application:

- ☐ New Project
- ☒ Continuing Review
- ☐ Modification

Type of Application Review:

- ☐ Full Board: Meeting Date:
- ☒ Expedited
- ☐ Exempt
- ☐ Facilitated

Approved for Populations:

- ☐ Children
  - ☐ Signature from one parent
  - ☐ Signature from two parents
- ☐ Prisoners
- ☐ Pregnant Women, Fetuses, Neonates
- ☐ Wards of State
- ☐ Decisionally Impaired

Criteria for approval are met per 45 CFR 46.111 and/or 21 CFR 56.111 as applicable.
Project determined to be minimal risk per 45 CFR 46.102(i) and/or 21 CFR 56.102(i) as applicable.

Source of Support:
NCI - National Cancer Institute
1RO1CA095614-01A1

MATERIALS APPROVED

This approval has been electronically signed by IRB Chair or Chair Designee:
Carissa Minder, BSN, RN
01/20/15 1353

660 South Euclid Ave., Campus Box 8089, St. Louis, MO 63110 Phone: (314) 633-7400 FAX: (314) 367-3041

IRB Approval: IRB approval indicates that this project meets the regulatory requirements for the protection of human subjects. IRB approval does not absolve the principal investigator from complying with other institutional, collegiate, or departmental policies or procedures.

Recruitment/Consent: Your IRB application has been approved for recruitment of subjects not to exceed the number indicated on your application form. If you are using written informed consent, the IRB-approved and stamped Informed Consent Document(s) are available in *my*IRB. The original signed Informed Consent Document should be placed in your research files. A copy of the Informed Consent Document should be given to the subject. (A copy of the *signed* Informed Consent Document should be given to the subject if your Consent contains a HIPAA authorization section.)

Continuing Review: Federal regulations require that the IRB re-approve research projects at intervals appropriate to the degree of risk, but no less than once per year. This process is called "continuing review." Continuing review for non-exempt research is required to occur as long as the research remains active for long-term follow-up of research subjects, even when the research is permanently closed to enrollment of new subjects and all subjects have completed all research-related interventions and to occur when the remaining research activities are limited to collection of private identifiable information. Your project "expires" at midnight on the date indicated on the preceding page ("Next IRB Approval Due on or Before"). You must obtain your next IRB approval of this project by that expiration date. You are responsible for submitting a Continuing Review application in sufficient time for approval before the expiration date, however you will receive reminder notice prior to the expiration date.

Modifications: Any change in this research project or materials must be submitted on a Modification application to the IRB for prior review and approval, except when a change is necessary to eliminate apparent immediate hazards to subjects. The investigator is required to promptly notify the IRB of any changes made without IRB approval to eliminate apparent immediate hazards to subjects using the Modification/Update Form. Modifications requiring the prior review and approval of the IRB include but are not limited to: changing the protocol or study procedures, changing investigators or funding sources, changing the Informed Consent Document, increasing the anticipated total number of subjects from what was originally approved, or adding any new materials (e.g., letters to subjects, ads, questionnaires).

Unanticipated Problems Involving Risks: You must promptly report to the IRB any unexpected adverse experience, as defined in the IRB/HRPO policies and procedures, and any other unanticipated problems involving risks to subjects or others. The Reportable Events Form (REF) should be used for reporting to the IRB.

Audits/Record-Keeping: Your research records may be audited at any time during or after the implementation of your project. Federal and University policies require that all research records be maintained for a period of seven (7) years following the close of the research project. For research that involves drugs or devices seeking FDA approval, the research records must be kept for a period of three years after the FDA has taken final action on the marketing application, if that is longer than seven years.

Additional Information: Complete information regarding research involving human subjects at Washington University is available in the "Washington University Institutional Review Board Policies and Procedures." Research investigators are expected to comply with these policies and procedures, and to be familiar with the University's Federalwide Assurance, the Belmont Report, 45CFR46, and other applicable regulations prior to conducting the research. This document and other important information is available on the HRPO website http://hrpohome.wustl.edu/.

National Cancer Institute
Central IRB Initiative

CIRB Operations Office
c/o: The EMMES Corporation
401 N. Washington St. Suite 700
Rockville, MD 20850
Tel: 1-888-657-3711 (Toll Free)
Fax: 301-560-6538
Email: ncicirbcontact@emmes.com

September 26, 2013

Matthew Ellis, MB, PhD, FRCP
Washington University School of Medicine
Barnes Jewish Hospital, Campus Box 8056
660 South Euclid Ave Section of Medical Oncology
St. Louis, MO 63110

Re: **CIRB Approval for Continuation of ACOSOG-Z1031**

A Randomized Phase III Trial Comparing 16 to 18 Weeks of Neoadjuvant Exemestane (25 mg daily), Letrozole (2.5 mg), or Anastrozole (1 mg) in Postmenopausal Women with Clinical Stage II and III Estrogen Receptor Positive Breast Cancer (Protocol Version Date 07/13/12)

Dear Dr. Ellis:

The NCI Adult Central IRB (CIRB)—Late Phase Emphasis has reviewed and approved the continuation of the above-referenced study (Protocol Version Date 07/13/12) for a period of 12 months. The continuing review of this study was conducted and approved in accordance with the Federally-defined categories of expedited review outlined in 45 CFR 46.110(b)(1) and 21 CFR 56.110(b)(1) Category 8(a) on September 25, 2013.

CIRB approval for this study will expire on September 24, 2014.

Accrual to the study is closed. A copy of the current consent form (Protocol Version Date 07/13/12) is provided for your records.

As the Study Chair, you are responsible for reporting study-related activity to the CIRB.

The CIRB complies with the Federal regulations 21 CFR 50, 21 CFR 56, and 45 CFR 46.

If you have any questions regarding this review, please contact the Adult CIRB Coordinator at adultcirb@emmes.com.

Sincerely,

Roy Smith, MD
Chair, NCI Adult Central IRB—Late Phase Emphasis

cc: John Olson, MD, PhD
Kelly Hunt, MD
Michael Kelly, MA
Tonya Haynes, MPH, RAC
Susan Budinger
Heather Becker
Geneviève Snyder