Factors Associated with Engraftment Success of Patient-Derived Xenografts of Breast Cancer

Jongwon Lee[1*], GunHee Lee[1*], Hye Seon Park[2], Byung-Kwan Jeong[1], Gyungyub Gong[1], Jae Ho Jeong[3], and Hee Jin Lee[1,2]

1Department of Pathology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, South Korea 2NeogenTC Corp., Seoul, South Korea3Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea * These authors contributed equally.

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BACKGROUND

- Establishing patient-derived xenograft (PDX) models remains a challenging and resource-intensive process, and the low engraftment rate of breast PDX models has been a major obstacle in preclinical studies.
- In this study, clinicopathologic factors and morphometric features extracted by artificial intelligence (AI) were analyzed to identify features associated with the success of PDX engraftment in breast cancer.

METHODS

- **Patient selection and data acquisition**: Surgically resected 372 breast tumor samples were collected from 2016 to 2021. Clinicopathological features, including neoadjuvant chemotherapy (NAC) status, pT and pN categories, immunohistochemically evaluated estrogen receptor (ER), progesterone receptor (PR) status, and HER2 status, Ki-67 labeling index (Ki-67LI)percentages were recorded for each tumor. Histological TIL levels were estimated by calculating the percentage of the area occupied by mononuclear inflammatory cells and plasma cells within the stromal area of the invasive carcinoma.
- **In Vivo Tumor Implantation**: Resected tumor specimens were orthotopically implanted into the 4th mammary fat pads of female NOD/SCID mice. Estrogen pellets were inserted for HR-positive subtype tumor. Tumor size was monitored until reaching 1 to 2 cm diameter (~2,000 mm^3 volume) before euthanasia, excision, and passaging.
- **AI-Assisted Morphometric Analysis**: An AI model was developed for morphometric analysis, trained on hematoxylin and eosin (H&E) stained WSIs from a separate set of 64 breast cancer patients. The ResNet50 architecture, pre-trained on ImageNet, was used with image augmentation applied to the training set. The model processed WSIs into 112 × 112 pixel non-overlapping patches, categorized into various tissue types including adipose tissue, background, necrosis, stroma, terminal ductal lobular unit (TDLU), and carcinoma (**Figure 1A-G**). Specialized segmentation training was performed on another set of 15 representative breast cancer WSIs to evaluate TILs, using segmentation with immune cell markers CD3, CD20, and CD79 used for lympohocyte detection, employed a combination of ResNet50 and DeepLabV3 Plus architectures (**Figure.1H-J**).

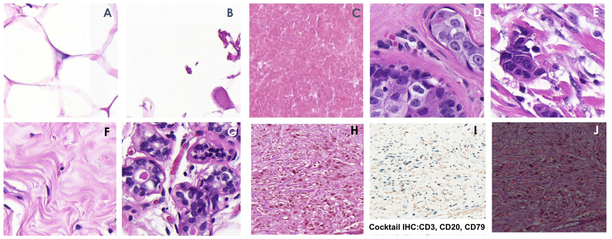

Figure 1. Artificial intelligence-assessed classification. A-G, Patch classification of tissues. **A**. Adipose; **B**. Background; **C.** Necrosis; **D**. Ductal carcinoma in situ, classified as carcinoma; **E**. Invasive carcinoma, classified as carcinoma; **F**. Stroma; **G**. Terminal ductal lobular unit; **H-J** Tumor-infiltrating lymphocytes classified with a segmentation model (X100, original magnification). **H**, TIL in H&E; **I**, Cocktail immunohistochemistry (IHC) for identification of lymphocytes, CD3, CD20, and CD79 cocktail IHC; **J**, red annotation indicating the area of cocktail IHC-stained lymphocytes in the H&E slides (annotated digitally processed image).

- **Intratumoral Proportions:** The AI model was them applied to 320 breast cancer cases used for PDX generation to identify patches within the boundaries of the carcinoma (**Figure 2**). Intratumoral proportions of specific tissue type including adipose tissue (AP), necrotic tissue (NP),TDLU (TDLUP), stroma (SP), and invasive carcinoma (ICP) were calculated. ICP was derived by multiplying the estimated intratumoral carcinoma patches by the pathologist-assessed true invasive carcinoma fraction.

- Tumor-infiltrating lymphocytes intratumoral proportion (TILP) was determined within the tumor boundary using a segmentation model, and a factor of 10,000 was applied to proportions

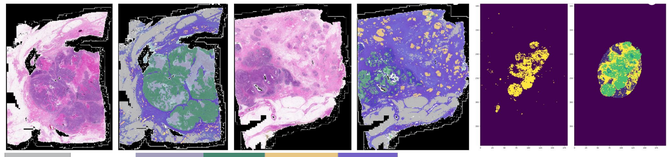

Figure 2. Representative successful (A) and failed (B) PDX graft cases and their corresponding AI-model patch classification. (**A**) Abundant intratumoral necrosis and carcinoma proportions in the H&E are also highlighted in sky-blue and green in the AI-model applied image, respectively (right); (**B**) Abundant stroma, and TDLU identified in the H&E slide (left) are also highlighted in the AI-classified image (**C**). The carcinoma patches (left side, yellow) were used to generate a tumor boundary (right side,green).

- **Statistical Analysis:** Various statistical analyses including independent sample t-tests, Chi-square tests, and logistic regression analyses were performed. The multivariate model retained variables that contributed to a lower Akaike Information Criterion (AIC) value. ROC curves and their corresponding area under the curve (AUC) were computed to assess the discriminative ability of the logistic regression models for predicting PDX engraftment. To evaluate reliability and stability, bootstrap analyses with 1,000 replicates were conducted to estimate the distribution of the AUC. Bias-corrected accelerated bootstrap methods were used to calculate confidence intervals for the AUC.

RESULTS

Table 1. Clinicopathologic characteristics associated with breast cancer PDX engraftment

Variable *		All (n=320) Failure (n=270)	All (n=320) Success (n=50)	Univariate ** (Odds ratio, 95% CI, p-value)	Multivariate ** (Odds ratio, 95% CI, p-value)
AP	Mean ± SD	639.9 ± 727.1	409.0 ± 484.0	**0.61 (0.47-0.79, p=.035)**	
NP	Mean ± SD	740.7 ± 981.9	1575.0 ± 1264.1	**1.58 (1.39-1.80, p<.001)**	**1.927 (1.077-3.449, p=0.027)**
TDLUP	Mean ± SD	504.4 ± 692.5	219.8 ± 233.3	**0.18 (0.08-0.41, p=.006)**	
SP	Mean ± SD	3377.5 ± 1265.9	2855.3 ± 1094.8	**0.63 (0.46-0.86, p=.007)**	1.545 (0.844-2.827, p=0.159)
TILP	Mean ± SD	17.8 ± 22.8	15.0 ± 15.9	0.00 (0-7833.4, p=0.401)	
ICP	Mean ± SD	4208.0 ± 1450.8	4571.6 ± 1201.5	1.19 (0.97-1.45, p=.097)	**1.820 (1.028-3.223, p=0.040)**
Age	Mean ± SD	50.9 ± 12.1	45.6 ± 10.4	**0.96 (0.94-0.99, p=.005)**	**0.96 (0.92-1.00, p=.032)**
Ki-67LI	Mean ± SD	41.2 ± 28.7	74.0 ± 14.3	**1.06 (1.04-1.08, p<.001)**	**1.05 (1.02-1.07, p<.001)**
Subtype	HER2+	20 (7.4%)	1 (2%)		
	HR+	130 (48.1%)	4 (8%)	0.62 (0.07-5.79, p=.671)	
	HR+/HER2+	30 (11.1%)	1 (2%)	0.67 (0.04-11.29, p=.779)	
	TNBC	90 (33.3%)	44 (88%)	**9.78 (1.27-75.23, p=.028)**	
Diagnosis	IDC	250 (92.6%)	45 (90%)		
	Other	20 (7.4%)	5 (10%)	1.39 (0.50-3.89, p=.532)	
HG	2	139 (51.5%)	3 (6%)		
	3	131 (48.5%)	47 (94%)	**16.62 (5.05-54.71, p<.001)**	**4.34 (1.08-17.53, p=.039)**
NAC	No	177 (65.6%)	12 (24%)		
	Yes	93 (34.4%)	38 (76%)	**6.03 (3.01-12.09, p<.001)**	**3.27 (1.41-7.60, p=.006)**
Size (cm)	Mean ± SD	3.1 ± 2.0	4.4 ± 2.8	**1.23 (1.09-1.38, p<.001)**	**1.20 (1.02-1.41, p=.029)**
LVI	No	151 (55.9%)	30 (60%)		
	Yes	119 (44.1%)	20 (40%)	0.85 (0.46-1.56, p=.594)	
Metastatic LNs	Mean ± SD	2.1 ± 5.8	4.5 ± 9.5	**1.04 (1.00-1.08, p=.031)**	
TIL (%)	Mean ± SD	12.0 ± 18.4	9.4 ± 13.8	0.99 (0.97-1.01, p=.351)	

AP, adipose proportion: NP, necrosis proportion: BP, background proportion: TDLUP, terminal ductal lobular unit proportion: SP, stroma proportion: TILP, tumor-infiltrating lymphocyte proportion;
ICP, invasive carcinoma proportion; Ki-67LI, Ki-67 labeling index (%): HR+, hormone receptor-positive breast cancer; TNBC, Triple-negative breast cancer; HR+/HER2+, hormone receptor and HER2 positive breast cancer;
HER2+, HER2 positive breast cancer; IDC, invasive ductal carcinoma; HG, histologic grade; NAC, Neoadjuvant chemotherapy; LVI, lymphovascular invasion; LN, lymph node; TIL, Tumor infiltrating lymphocytes
* AP, BP, NP, TDLUP, SP, TILP, ICP values scaled up by 10,000
** AP,BP,NP,TDLUP,SP, TILP,ICP: OR calculated with 1,000-unit change, corresponding to 0.1% of intratumoral percentage adjustment
*** Bold: significant at P < .05.

- In primary breast cancer, multivariate logistic regression analyses with the lowest AIC value demonstrated that high Ki-67LI (p<0.001), younger age at diagnosis (p=0.032), post-NAC (p=0.006), higher histologic grade (p=0.039), larger tumor size (p=0.029), and AI-assessed higher NP (p=0.027) and ICP (p=0.040) were significant factors for successful PDX engraftment (**Table 1** 1, AUC: 0.905, **Figure 3A**).

Table 2. Clinicopathologic characteristics associated with breast cancer PDX engraftment in NAC group

Variable *		NAC group (n=131) Failure (n=93)	NAC group (n=131) Success (n=38)	Univariate ** (Odds ratio, 95% CI, p-value)	Multivariate** (Odds ratio, 95% CI, p-value)
AP	Mean ± SD	517.3 ± 622.2	450.9 ± 528.0	0.82 (0.74-0.91, p=.562)	
NP	Mean ± SD	901.3 ± 1101.4	1642.8 ± 1358.9	**1.60 (1.38-1.85, p=.003)**	
TDLUP	Mean ± SD	475.2 ± 730.2	201.6 ± 213.9	**0.22 (0.18-0.27, p=.034)**	0.181 (0.033-1.00, p=0.06)
SP	Mean ± SD	3512.4 ± 1523.4	2855.7 ± 1180.3	**0.71 (0.60-0.84, p=.022)**	
TILP	Mean ± SD	10.2 ± 14.0	11.3 ± 13.3	283.00 (29.48-2718.95, p=.676)	
ICP	Mean ± SD	4105.8 ± 1552.8	4505.6 ± 1187.3	1.00 (1.00-1.00, p=.159)	
Age	Mean ± SD	49.6 ± 10.3	45.6 ± 11.0	0.96 (0.93-1.00, p=.053)	
Ki-67LI	Mean ± SD	39.2 ± 32.5	75.0 ± 14.3	**1.06 (1.03-1.09, p<.001)**	**1.07 (1.03-1.10, p<.001)**
Subtype	HER2+	8 (8.6%)	1 (2.6%)		
	HR+	35 (37.6%)	3 (7.9%)	0.69 (0.06-7.48, p=.757)	
	HR+/HER2+	14 (15.1%)	1 (2.6%)	0.57 (0.03-10.43, p=.706)	
	TNBC	36 (38.7%)	33 (86.8%)	7.33 (0.87-61.82, p=.067)	
Diagnosis	IDC	87 (93.5%)	34 (89.5%)		
	Other	6 (6.5%)	4 (10.5%)	1.71 (0.45-6.42, p=.430)	
HG	2	41 (44.1%)	3 (7.9%)		
	3	52 (55.9%)	35 (92.1%)	**9.20 (2.64-32.05, p<.001)**	
Miller-Payne grade	Mean ± SD	2.3 ± 0.8	1.6 ± 0.8	**0.34 (0.20-0.57, p<.001)**	**0.30 (0.16-0.58, p<.001)**
RCB score	Mean ± SD	3.1 ± 1.0	3.4 ± 1.2	1.24 (0.86-1.78, p=.253)	
Size (cm)	Mean ± SD	4.0 ± 2.6	5.0 ± 2.9	1.13 (0.99-1.30, p=.064)	
LVI	No	44 (47.3%)	21 (55.3%)		
	Yes	49 (52.7%)	17 (44.7%)	0.73 (0.34-1.55, p=.410)	
Metastatic LNs	Mean ± SD	4.1 ± 8.9	5.8 ± 10.6	1.02 (0.98-1.06, p=.350)	
TIL (%)	Mean ± SD	6.1 ± 11.6	6.3 ± 8.5	1.00 (0.97-1.04, p=.928)	

AP, adipose proportion: NP, necrosis proportion; BP, background proportion: TDLUP, terminal ductal lobular unit proportion: SP, stroma proportion: TILP, tumor-infiltrating lymphocyte proportion;
ICP, invasive carcinoma proportion; Ki-67LI, Ki-67 labeling index (%): HR+, hormone receptor-positive breast cancer; TNBC, Triple-negative breast cancer; HR+/HER2+, hormone receptor and HER2 positive breast cancer;
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* AP, BP, NP, TDLUP, SP, TILP, ICP values scaled up by 10,000
** AP,BP,NP,TDLUP,SP, TILP,ICP: OR calculated with 1,000-unit change, corresponding to 0.1% of intratumoral percentage adjustment
*** Bold: significant at P < .05.

- In the NAC group, a higher Ki-67LI (p<0.001), lower Miller-Payne grade (p<0.001), and reduced proportion of TDLUP(p=0.06) collectively provided excellent prediction accuracy for successful PDX engraftment (**Table 2**, AUC: 0.89, **Figure 3C**).

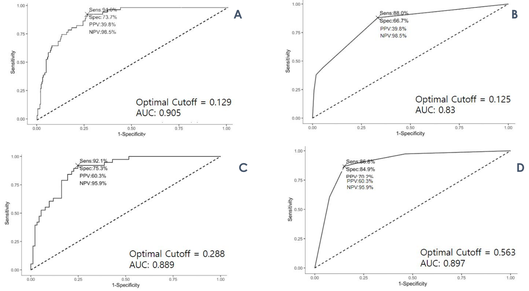

Figure 3. Receiver-operated curves for predictive models of engraftment success. (**A**) Multivariate logistic regression of primary breast cancers, incorporating the selected variables from both AI-evaluated morphometric features and clinicopathological findings. (**B**) Pruned decision tree prediction model using clinicopathological and AI-derived morphometric features for primary breast cancers. (**C**) Multivariate logistic regression, incorporating the selected variables from both AI-evaluated morphometric features and clinicopathological findings. (**D**) Pruned decision tree prediction model in the NAC group, utilizing both clinicopathological and AI-derived morphometric features.

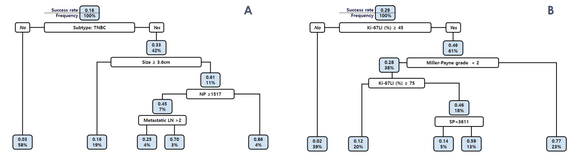

Figure 4. Pruned decision tree models for PDX engraftment success prediction.
(**A**) All primary breast cancers; (**B**) NAC group.

- We performed decision tree analyses to predict PDX engraftment successes. In the all breast cancer patient group, The model achieved an AUC of 0.8304 and a PPV of 0.398 and NPV of 0.985 at the optimal cutoff point (**Figure 3B**). TNBC subtype, tumor size, NP, Ki-67LI and patient age were the most important variables in the model (**Figure 4A**).
- In NAC-treated primary breast cancer, the model achieved an AUC of 0.8967 and a PPV of 0.6034 and NPV of 0.9589 at the optimal cutoff point (**Figure 3D**). Ki-67LI, Miller-Payne grade, subtype、SP, and histologic grade were the most important variables in the model (**Figure 4B**).

CONCLUSION

- In this study, we found that higher Ki-6LI, younger age at diagnosis, NAC treatment status, larger tumor size, higher histologic grade, and higher proportions of intratumoral necrosis and invasive carcinoma were significant variables predictive of PDX engraftment success. Also, lower Miller-Payne grade and reduced proportion of TDLUP(p=0.06) were important factors for PDX engraftment in NAC group.
- Subtype TNBC and SP also were notable factors in the decision tree analyses.
- Our study demonstrated a logarithmic increase in the success rates PDX grafts through repeated passagesm which may be ascribed to genetic drift.
- Elevated Ki-67LI, a marker of cellular proliferation stood out as a pivotal factor for PDX engraftment success in the current study. Also, high Ki-67LI, high histologic grade and the TNBC subtype often co-exist in breast cancerm which are known to have increased proportions of cancer stem cells.
- This cluster of associated variables is notably evident in younger patient populations, who commonly present with high-grade TNBC tumors,
- Therefore, the high rates of PDX engraftment in our study could be attributed to the co-existence of these interrelated variables, each contributing to the tumor's aggressive nature and adaptability to a xenograft environment.

CONTACTS

- Jongwon Lee, M.D., alyssa8921@gmail.com
- GunHee Lee, Ph.D., gun9103@gmail.com
- Hee Jin Lee, M.D., Ph.D., backlila@gmail.com