Supplementary appendix

A phase II study of durvalumab and tremelimumab with front-line neoadjuvant chemotherapy in patients with advanced-stage ovarian cancer: primary analysis in the original cohort of KGOG3046/TRU-D

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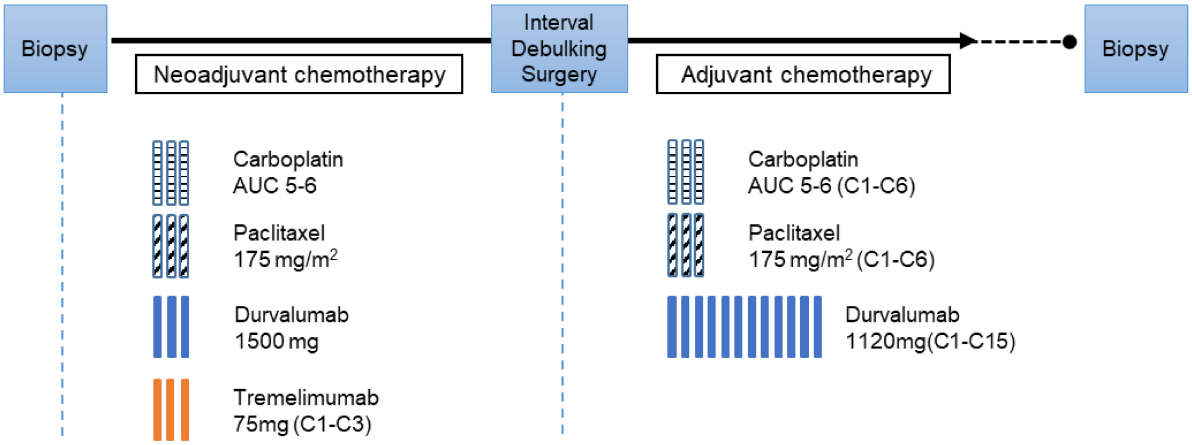
Supplementary Table S1. Postoperative complications

	Patients (n=23)				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Wound dehiscence	0	0	1 (4.4%)	0	0
Wound infection	0	0	1 (4.4%)	0	0
Abdominal pain	0	4 (17.4%)	1 (4.4%)	0	0
Lymphedema	1 (4.4%)	0	0	0	0
Ileus	1 (4.4%)	0	2 (8.7%)	0	0
Bowel perforation	0	0	1 (4.4%)	0	0
Urinary retention	0	0	0	0	0

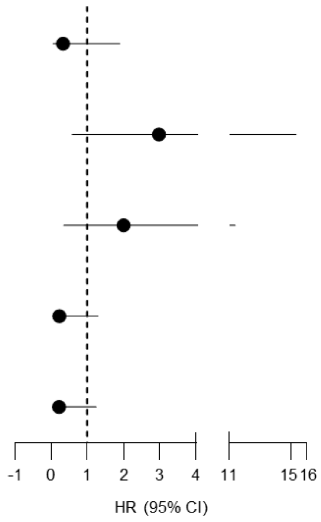
Supplementary Figure S1. Study design



Supplementary Figure S2. Forest plot of hazard ratios for progression-free survival (A) and overall survival (B) according to baseline characteristics and neoadjuvant period outcomes. CRS, chemotherapy response score; NA, not analyzed; NR, not reached

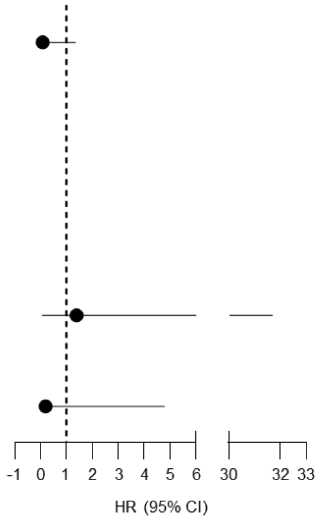
A

Variable	Events/Patients	HR (95% CI)	Median PFS (95% CI)	*P*-value
Age (years)				0.558
≤60	10/17	0.33 (0.06, 1.90)	25.33 (6.76, 43.91)	
>60	3/6	Reference	10.81 (7.35, 14.27)	
***BRCA1/2* status**				0.835
mutated	4/7	2.98 (0.58, 15.34)	25.33 (15.09, 35.57)	
non-mutated	8/15	Reference	13.70 (0, 30.34)	
Stage				0.485
IIIC	2/3	2.00 (0.35, 11.41)	11.14 (4.51, 17.76)	
IV	11/20	Reference	25.33 (5.41, 45.26)	
R0 resection				0.034
Yes	9/17	0.23 (0.04, 1.30)	32.13 (18.69, 45.58)	
No	4/6	Reference	12.62 (8.99, 16.24)	
CRS				0.080
3	4/9	0.22 (0.04, 1.25)	32.13 (NA, NA)	
1/2	9/14	Reference	12.62 (9.42, 15.81)	

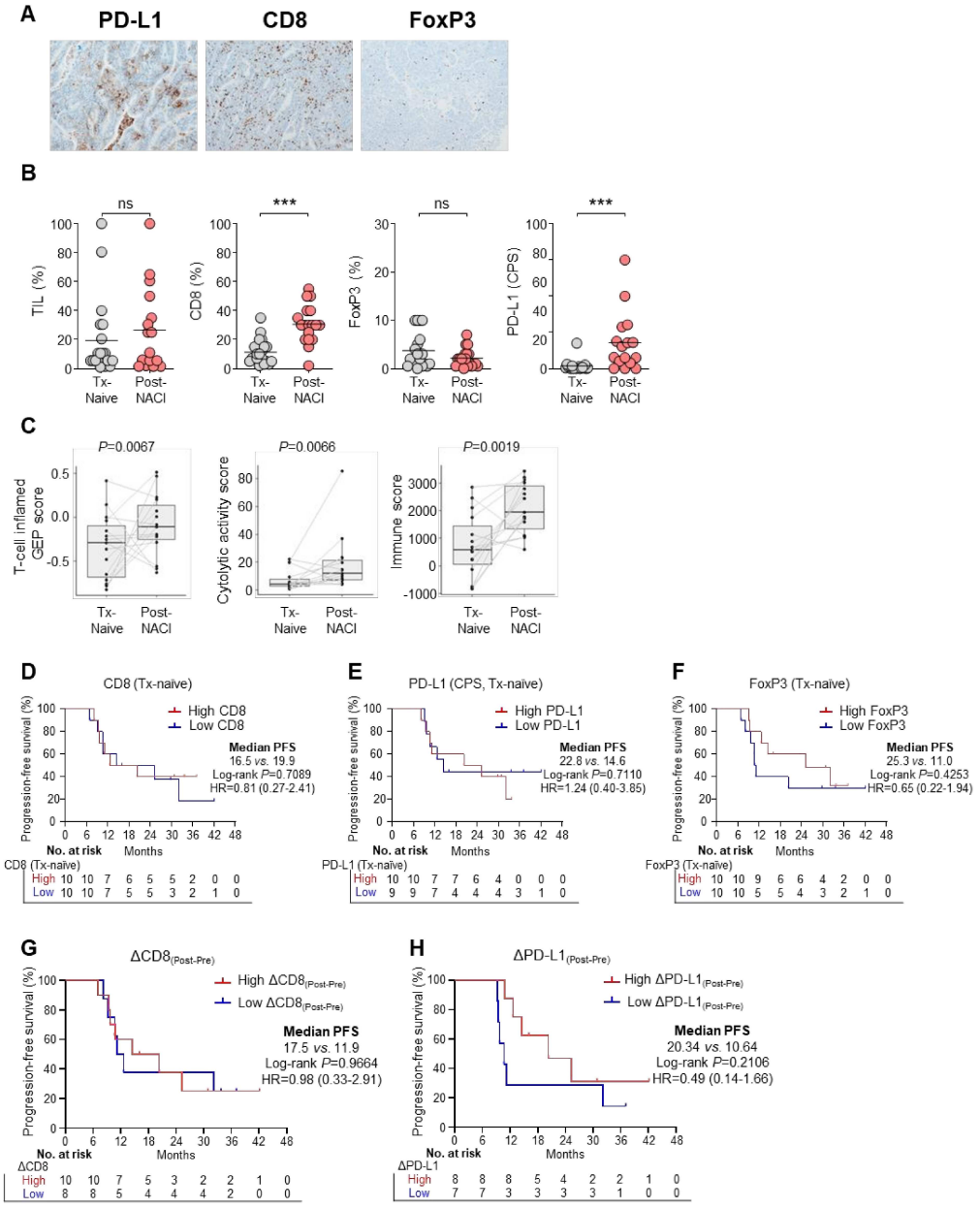


B

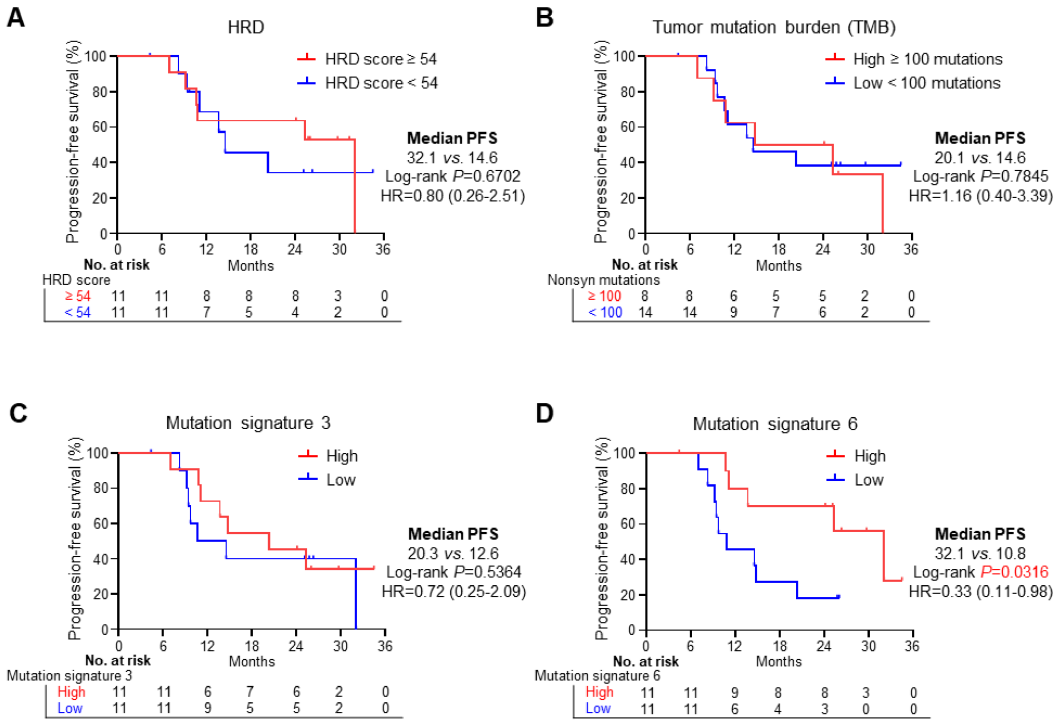
Variable	Events/Patients	HR (95% CI)	Median OS (95% CI)	*P*-value
Age (years)				0.069
≤60	1/17	0.08 (0.01, 1.35)	NR	
>60	2/6	Reference	27.83 (13.74, 41.92)	
***BRCA1/2* status**				0.265
mutated	0/7	NA	NR	
non-mutated	3/15		NR	
Stage				0.599
IIIC	0/3	NA	NR	
IV	3/20		NR	
R0 resection				0.601
Yes	2/17	1.34 (0.06, 31.69)	NR	
No	1/6	Reference	NR	
CRS				0.490
3	1/9	0.19 (0.01, 4.79)	NR	
1/2	2/14	Reference	NR	



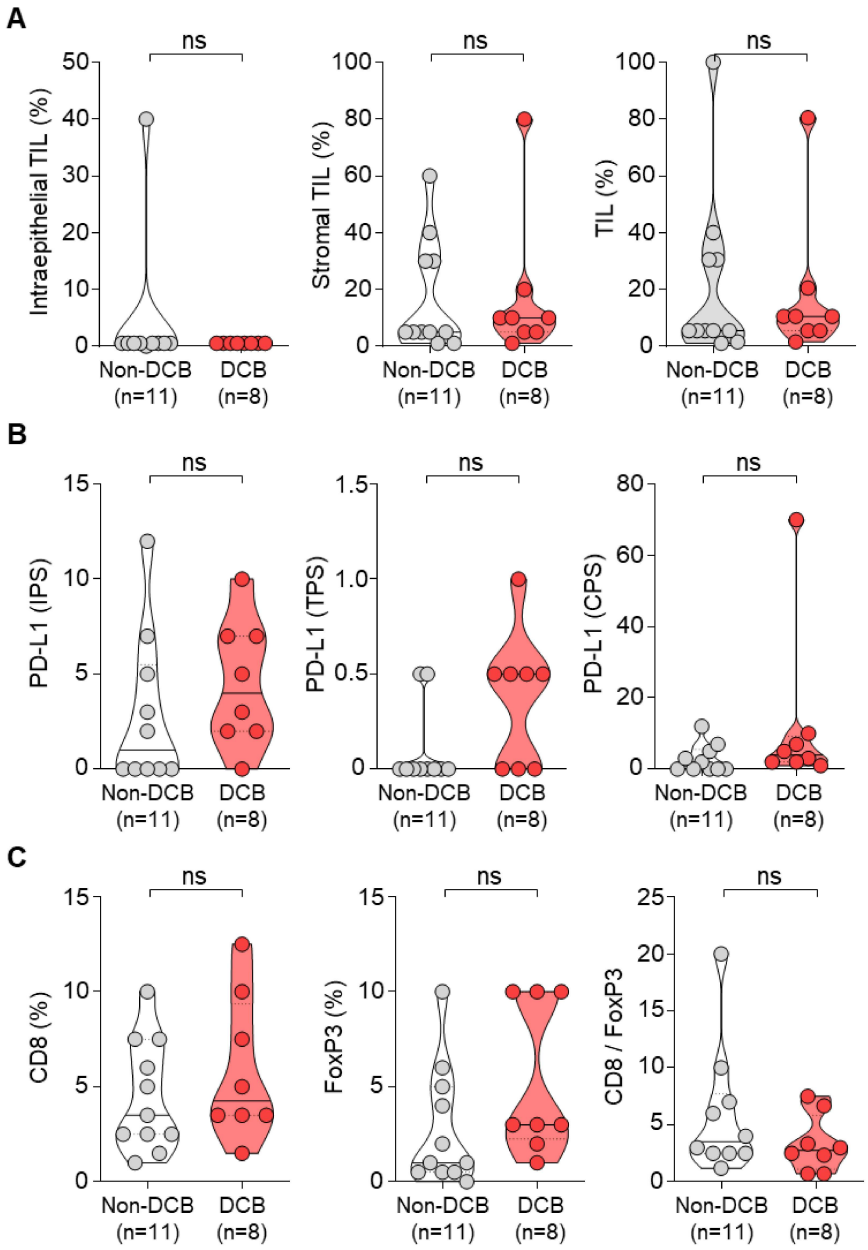
Supplementary Figure S3. Comparison of immune characteristics of treatment-naïve versus post-NACI tumor tissues. (A–B) Immunohistochemistry analysis for tumor-infiltrating lymphocyte (TIL), PD-L1, CD8, and FoxP3 expression in treatment-naïve and post-NACI tumor tissues. (C) Comparison of gene expression profiles (GEP) between treatment-naïve and post-NACI tumor tissue in terms of T-cell inflamed GEP score, cytolytic activity score, and immune score. (D–F) Comparison of progression-free survival according to the pre-treatment levels of CD8, PD-L1 and FoxP3. (G, H) Comparison of progression-free survival according to the changes in CD8 and PD-L1 levels. NACI, neoadjuvant chemo-immunotherapy



Supplementary Figure S4. Progression-free survival according to (A) homologous recombination deficiency (HRD) status, (B) tumor mutation burden (TMB), (C) mutational signature 3, and (D) mutational signature 6.

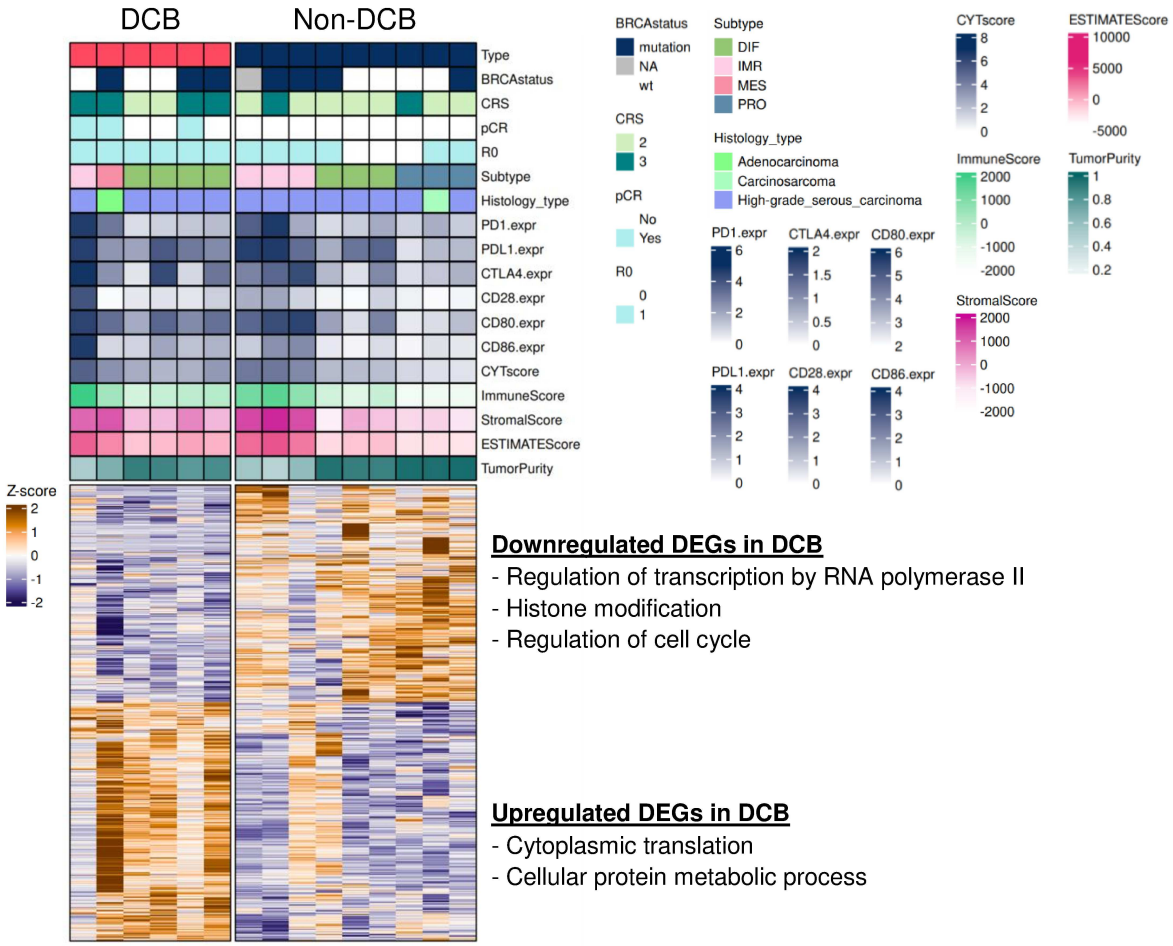


Supplementary Figure S5. Immunohistochemistry analysis for tumor-infiltrating lymphocyte, PD-L1, CD8, and FoxP3 expression in pretreatment tumors between DCB and non-DCB patients. Comparison of (A) lymphocytes infiltration, (B) PD-L1, (C) CD8, and FoxP3 expression between non-DCB and DCB patients. TIL, tumor-infiltrating lymphocyte; DCB, durable clinical benefit; IPS, immune proportion score; TPS, tumor proportion score; CPS, combined positive score



Supplementary Figure S6. Transcriptome analysis of pretreatment tumors between DCB and non-DCB patients.

DCB, durable clinical response; CRS, chemotherapy response score; pCR, pathologic complete response; R0, no residual disease; DIF, differentiated, IMR; immunoreactive; MES, mesenchymal; PRO, proliferative; DEG, differentially expressed gene



Downregulated DEGs in DCB

- Regulation of transcription by RNA polymerase II
- Histone modification
- Regulation of cell cycle

Upregulated DEGs in DCB

- Cytoplasmic translation
- Cellular protein metabolic process

Supplementary Methods

Immunohistochemistry

IHC was performed using formalin-fixed, paraffin-embedded tissue blocks that were sectioned at 4 μm thickness. After deparaffinization in xylene and rehydration through graded alcohol, samples were processed with an automatic immunostaining instrument (Ventana Benchmark XT; Ventana Medical Systems). Antigen retrieval was performed using a Cell Conditioning Solution (CC1; Ventana Medical Systems). The sections were incubated with antibodies against PD-L1 (prediluted, clone SP263, Ventana Medical Systems), PD-L1 (prediluted, clone 22C3, Dako, Glostrup, Denmark), CD8 (prediluted, clone C8/144B, Dako, Glostrup, Denmark), and FOXP3 (1:50, clone 236A/E7, Abcam, Cambridge, UK). The level of tumor-infiltrating lymphocytes (TILs) was assessed following the guidelines proposed by the International TILs Working Group in 2014[1]. Depending on the lymphocyte-infiltrated sites, TILs were classified as infiltrated lymphocytes in the tumor stroma (sTILs) or the tumor cell islets (iTILs). The percentage of the stromal TILs was calculated as the percentage of the area occupied by mononuclear inflammatory cells over the total intratumoral stromal area. For PD-L1 expression, the combined positive score (CPS) for clone 22C3 and tumor proportion score (TPS) for clone SP263 were calculated as previously described[2]. The IHC stain was scored and interpreted by an expert pathologist (E Park).

Sample preparation for sequencing

Pretreatment tumor samples from fresh-frozen tissues and matched peripheral blood samples were obtained for whole-exome sequencing. In addition, post-NACI tumors and matched blood samples were acquired to observe immunologic changes during treatment. After tumor cellularity was reviewed by an expert pathologist (E Park), tumor DNA and RNA were extracted from tumor tissues using ALLPrep DNA/RNA mini kit (Qiagen, USA) according to

the manufacturer's protocol. We measured genomic DNA and RNA concentration, purity, and integrity using a Nanodrop 8000 UV-Vis spectrometer (Thermo Scientific Inc., DE, USA), Qubit 2.0 Fluorometer (Life Technologies Inc., Grand Island, NY, USA), and the Agilent 4200 TapeStation.

Whole exome and transcriptome sequencing

Whole transcriptome sequencing was carried out using the library constructed with 500 ng genomic RNA from each sample. The library construct was performed using a Truseq RNA sample preparation v2 kit (Illumina, USA) according to the manufacturer's protocol. To obtain a paired-end RNA sequencing library, the cDNAs were synthesized from total RNA via a reverse transcription reaction with poly (dT) primers using SuperScriptTM II reverse transcriptase (Invitrogen/Life Technologies, Grand Island, NY, USA). After synthesized cDNA, end repair of cDNA fragments, the addition of deoxyadenine base to 3′ ends, ligation of adapters, and PCR amplification were conducted. The quantity and quality of the library were measured and sequencing of the transcriptome library was carried out.

Processing whole transcriptome sequences

Sequencing reads were aligned to the human reference genome (GRCh37) by using STAR aligner[3]. Expected counts of reads mapped into genes were estimated and transformed into TPM values by RSEM[4]. The T-cell–inflamed GEP score was derived from an 18-gene signature measured based on the previous study[5]. Molecular subtypes were determined using consensusOV classifier that represents the subtype classifications for ovarian cancers (http://bioconductor.org/packages/consensusOV).

References

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2 Paver EC, Cooper WA, Colebatch AJ*, et al.* Programmed death ligand-1 (PD-L1) as a predictive marker for immunotherapy in solid tumours: a guide to immunohistochemistry implementation and interpretation. *Pathology* 2021;53:141-56.

3 Dobin A, Davis CA, Schlesinger F*, et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013;29:15-21.

4 Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 2011;12:323.

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