Molecular profiling of testicular cancer

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Updated Abstract

Background: The incidence of testicular germ cell tumors (TGCT) has been increasing in many western countries for several decades. For 2013, about 7900 new TGCT cases with 370 deaths are estimated in the United States. While the overall prognosis in metastatic TGCT is excellent, treatment options for patients (pts) relapsing with cisplatin-refractory disease are limited. A personalized approach to therapy based upon molecular diagnostic tools to predict response to specific therapeutic agents may help pts in whom standard chemotherapy has failed.
Methods: See below
Results: In the cohort of pts with advanced / refractory TGCT, the most commonly observed changes in protein expression were upregulation of TOPO2A (87%), EGFR (86%), RRM1 (60%), SPARC (40%), and TLE3 (40%), and downregulation of ERCC1 (83%), MGMT (69%), and PTEN observed in 65% of patients. Amplification of EGFR, cMET, and HER2 was observed in 19%, 14%, and 5% of cases, respectively. In 10 cases analyzed by NGS, the most common mutation involved TP53 (30%) followed by KRAS (10%), cKIT (10%) and SMAD4 (10%) mutations. No NRAS or HRAS mutations were found. One patient with a KRAS mutation also had an activating cKIT mutation. All 3 pts with TP53 mutations did not have a loss of PTEN protein expression but no other mutations. Pathway profiling revealed that with the exception of loss of PTEN, 90% of patients were predominantly ERK and mTOR pathway wildtype, and activation of the ERK pathway only occurred through a single mutation in the KRAS gene.
Conclusions: A low prevalence of mutations was observed in advanced / refractory TC. Predictive biomarkers may be used to guide treatment decisions. Targeting AKT signaling, or the cKIT, HER2, or cMET pathways may warrant treatment options in selected pts.

Background

- Despite the high cure rates of GCT patients through cisplatin-based standard combination chemotherapy with or without secondary resection of residual masses, approximately 20% of pts do not respond expectedly to cisplatin-based chemotherapy and long-term survival is rarely achieved in cisplatin-refractory disease[1,2].
- Both the mechanisms of the unique sensitivity of GCTs to cisplatin-based chemotherapy and of the rare state of treatment resistance are scarcely understood[3,4].
- Several clinical trials investigating molecularly targeted drugs have been conducted, but results were unsatisfactory.
- The purpose of this analysis was o better define the molecular profile and thereby to detect potential targets for the treatment of refractory GCTs.

Methods

- Data from Caris Life Sciences were made available for expert clinician interpretation. Eighty-eight cases of testicular neoplasms (TN) referred to Caris Life Sciences between 2009 and March 2014 were evaluated; diagnoses were collected from referring physicians and classified at intake based on pathology and clinical history. 51 pts (58%) had a TN of germ cell origin, 8 (9%) had stromal tumors, and 26 (23%) had TN of other origin (i.e. sarcoma, NOS, adenocarcinoma).
- Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC), gene amplification (CISH or FISH), and/or RNA fragment analysis.
- IHC analysis was performed on formalin-fixed paraffin-embedded tumor samples using commercially available detection kits, automated staining techniques (Benchmark XT, Ventana, and AutostainerLink 48, Dako), and commercially available antibodies.
- Fluorescent in-situ hybridization (FISH) was used for evaluation of the HER-2/neu [HER-2/CEP17 probe], EGFR [EGFR/CEP7 probe], and cMET [cMET/CEP7 probe] (Abbott Molecular/Vysis). HER-2/neu and cMET status were evaluated by chromogenic in-situ hybridization (INFORM HER-2 Dual ISH DNA Probe Cocktail; commercially available cMET and chromosome 7 DIG probe; Ventana). The same scoring system was applied as for FISH.
- Direct sequence analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using the Illumina MiSeq platform. Specific regions of 45 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel.
- Mutation analysis by Sanger sequencing included selected regions of BRAF, KRAS, c-KIT, EGFR, and PIK3CA genes and was performed by using M13-linked PCR primers designed to amplify targeted sequences.
- In Caris Molecular Intelligence™ (CMI) reports provided to the ordering physician after comprehensive tumor profiling, treatments associated with benefit were found in 100% of patients.
- Immunohistochemistry provided 100% of patients (51/51) with a treatment associated with benefit (with a median of 6 biomarkers linked to positive predictive associations per patient; range 2-9) compared to biomarkers measured by ISH providing a treatment associated with benefit in 9.8 % of cases tested (5/51). NGS found a mutation in 40% of tumors tested (4/10) compared to Sanger sequencing with a mutation found in 5.6% of tumors tested (1/18).
- Statistical analysis (unpaired t-tests used to compare biomarker expression across histologic subtypes) performed using GraphPad™.

Demographics

- No clinical data on disease stage, recurrence or prior treatment history was collected for these samples.
- TGCT separate roughly into seminomas and nonseminomas.
- Seminomas are extremely sensitive to chemotherapy and refractory disease is a rare phenomenon, which may reflect the low number of seminomatous TGCTs in this recent analysis. Stromal tumors of the testes are found even less often.

Germ Cell Testicular Cancer (n=51)	51
NSGCT	46
Germ Cell	*16*
Germ Cell Mixed	*10*
Choriocarcinoma	*9*
Embryonal	*5*
Yolk Sac	*5*
Teratoma	*1*
SGCT	5
Stromal Cell Testicular Cancer	8

N = number of pts; NSGCT nonseminomatous germ cell tumor; SGCT seminomatous germ cell tumor

Tumor Profiling of Testicular Germ Cell Cancer

- To define molecular alterations and detect potential therapeutic targets protein expression analysis by immunohistochemistry and gene amplification analysis by FISH were applied.
- A small subset of the overall population may be sensitive to either cKIT, cMET, or HER2 inhibition.
  - cMET protein overexpression and gene amplification were observed in two patients
  - HER2 protein overexpression co-existed with gene amplification in one patient .
- Patients frequently have MGMT or PTEN loss which may suggest sensitivity to temozolomide or mTOR

Germ Cell Testicular Cancer (n=51)	Biomarker	Platform	Threshold
0% (0/48)	AR	IHC	*=0+ or <10% or ≥1+ and ≥10%*
32.3% (10/31)	cKIT	IHC	*=0+ and =100% or ≥2+ and ≥30%*
5.3% (1/19)	cMET	IHC	*<50% or <2+ or ≥2+ and ≥50%*
85.7% (6/7)	EGFR	IHC	*=0+ or <10% or ≥1+ and ≥10%*
0% (0/48)	ER	IHC	*=0+ or <10% or ≥1+ and ≥10%*
83.3% (25/30)	ERCC1 Loss	IHC	*<2+ or ≤3+ and <10% or =2+ and <50% or ≥3+ and ≥10% or ≥2+ and ≥50%*
4.3% (2/47)	HER2	IHC	*≤1+ or =2+ and ≤10% or ≥3+ and >10%*
68.9% (31/45)	MGMT Loss	IHC	*=0+ or ≤35% or ≥1+ and >35%*
20% (9/45)	PGP	IHC	*=0+ or <10% or ≥1+ and ≥10%*
4.2% (2/48)	PR	IHC	*=0+ or <10% or ≥1+ and ≥10%*
65.2% (30/46)	PTEN Loss	IHC	*=0+ or ≤50% or ≥1+ and >50%*
60% (27/45)	RRM1 Loss	IHC	*=0+ or <50% or <2+ or ≥2+ and ≥50%*
40% (20/50)	SPARC	IHC	*<30% or <2+ or ≥2+ and ≥30%*
40% (8/20)	TLE3	IHC	*<30% or <2+ or ≥2+ and ≥30%*
39% (16/41)	TOPO1	IHC	*=0+ or <30% or <2+ or ≥2+ and ≥30%*
87% (40/46)	TOP2A	IHC	*=0+ or <10% or ≥1+ and ≥10%*
55.6% (25/45)	TS Loss	IHC	*=0+ or ≤3+ and <10% or ≥1+ and ≥10%*
43.8% (7/16)	TUBB3 Loss	IHC	*<30% or <2+ or ≥2+ and ≥30%*
14.3% (1/7)	cMET	FISH	*Positivity for increased gene copy number by FISH has been defined as ≥ 5 copies in lung tumor cells. The gene copy number threshold for other tumor types has not been determined.*
18.8% (3/16)	EGFR	FISH	*Positivity for increased gene copy number by FISH has been defined as ≥4 copies in 40% or more tumor cells. Gene amplification is defined by the presence of a gene/chromosome per cell raio of ≥2, or ≥15 copies per cell in ≥10% of analyzed cells*
5% (1/20)	HER2	FISH	*HER2/Neu:CEP 17 signal ratio of ≥ 2.0; and non-amplification as <2.0 as per Ventana INFORM HER2 CISH Package insert*
10% (1/10)	cKIT	NGS	*Amino acids 42-101, 493-592, 632-745 and 806-866*
10% (1/10)	KRAS	NGS	*Amino acids 1-31, 38-71 and 97-150*
30% (3/10)	TP53	NGS	*Amino acids 1-20, 60-121, 126-307 and 322-346*

Comparison of Seminoma and Non-seminoma Germ Cell Tumors with Stromal Cell Tumors

- Seminoma and non-seminomatous GCT had relatively comparable results for many of the biomarkers tested.
- All 5 seminomas had loss of ERCC1, MGMT, PTEN and TS (TS loss was significant (p=0.0343).
- Stromal cancers had significantly more AR (p=0.0035) and PR (p<0.0001) expression and RRM loss (p=0.0401) than germ cell cancers. They also had total lack of TOP2A expression (p<0.0001) compared to germ cell tumors.

Seminoma (n=5)	Non-Seminoma Germ Cell (n=46)	Stromal (n=8)	Biomarker	Platform
0% (0/5)	0% (0/43)	20% (1/5)	AR	IHC
0% (0/1)	5.6% (1/18)	0% (0/1)	cMET	IHC
0% (0/5)	0% (0/43)	0% (0/5)	ER	IHC
100% (4/4)	80.8% (21/26)	66.7% (4/6)	ERCC1 Loss	IHC
0% (0/5)	4.8% (2/42)	0% (0/5)	HER2	IHC
100% (5/5)	65% (26/40)	60% (3/5)	MGMT Loss	IHC
0% (0/5)	22.5% (9/40)	60% (3/5)	PGP	IHC
0% (0/5)	4.7% (2/43)	60% (3/5)	PR	IHC
100% (5/5)	61% (25/41)	60% (3/5)	PTEN Loss	IHC
60% (3/5)	60% (24/40)	100% (7/7)	RRM1	IHC
40% (2/5)	40% (18/45)	40% (2/5)	SPARC	IHC
0% (0/1)	42.1% (8/19)	0% (0/1)	TLE3	IHC
20% (1/5)	41.7% (15/36)	33.3% (2/6)	TOPO1	IHC
60% (3/5)	90.2% (37/41)	0% (0/7)	TOP2A	IHC
100% (5/5)	50% (20/40)	71.4% (5/7)	TS Loss	IHC
0% (0/1)	46.7% (7/15)	0% (0/1)	TUBB3 Loss	IHC

Comparison of Biopsy Site (Primary vs Metastatic)

- Many of the biomarkers tested show comparable levels of expression, independent of the site of biopsy which was analysed ,with no significant differences observed.

Testes Biopsy (n=9)	Biopsy - All other sites (n=43)	Biomarker	Platform
0% (0/9)	0% (0/39)	AR	IHC
0% (0/5)	7.1% (1/14)	cMET	IHC
0% (0/9)	0% (0/39)	ER	IHC
100% (4/4)	80.8% (21/26)	ERCC1 Loss	IHC
0% (0/9)	5.3% (2/38)	HER2	IHC
88.9% (8/9)	63.9% (23/36)	MGMT Loss	IHC
0% (0/8)	24.3% (9/37)	PGP	IHC
0% (0/9)	5.1% (2/39)	PR	IHC
77.8% (7/9)	62.2% (23/37)	PTEN Loss	IHC
50% (4/8)	62.2% (23/37)	RRM1	IHC
33.3% (3/9)	41.5% (17/41)	SPARC	IHC
40% (2/5)	40% (6/15)	TLE3	IHC
37.5% (3/8)	39.4% (13/33)	TOPO1	IHC
87.5% (7/8)	86.8% (33/38)	TOP2A	IHC
62.5% (5/8)	54.1% (20/37)	TS Loss	IHC
50% (2/4)	41.7% (5/12)	TUBB3 Loss	IHC

All other biopsy sites included Lymph Node (8), Lung&Bronchus (7), Retroperitoneum&Peritoneum (6), Connective & Soft Tissue (4), Bones and Joints (3), Brain & Spinal Cord (3), Liver (1), Neck (1) Pelvis (1), Other (5)

Sequencing of Testicular Germ Cell Tumors by NGS, Sanger and PCR analysis

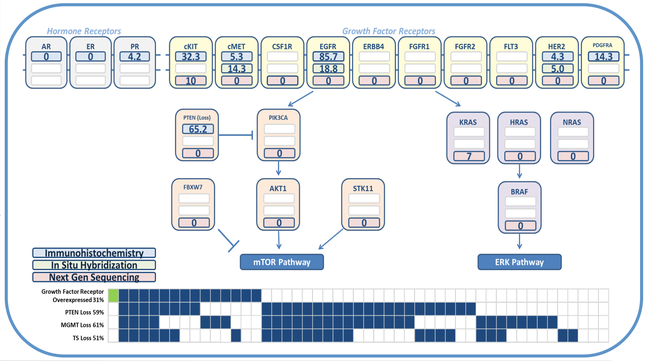
- NGS using a 44 gene panel was performed in 10 patients and only 6 mutations were found in 4 patients.
- 3 of these were in TP53 gene indicated that this may be an important oncogenic driver in tumorigenesis. All 3 TP53 patients did not have PTEN loss.
- Co-existing mutations in cKIT (D816Y, an activating mutation in the kinase domain) and KRAS were found in a single tumor.

	NGS		Sanger		PCR		Total Incidence (%)
	Mutations found	*Patients tested*	*Mutations found*	*Patients tested*	*Mutations found*	*Patients tested*	
cKIT	1	10	0	2	0	0	8.3
KRAS	1	10	0	16	1	2	7.1
SMAD4	1	10	0	0	0	0	10
TP53	3	10	0	0	0	0	30

- Although PIK3CA mutations were not observed in this cohort, a single patient with a germ cell cancer had PIK3CA amplification without PTEN loss.

Overview of Detected Pathway Alterations in Testicular Germ Cell Cancer

- The table below shows the overlap of growth factor receptor overexpression with loss of PTEN, MGMT and TS. Dark blue indicates a protein alteration, green representing two alterations.
- PTEN loss is frequently observed, especially when there is no overexpression of receptors. This loss in PTEN expression, and thus loss of tumor suppressor function in the mTOR pathway, tends to occur in combination with loss of MGMT or TS. This may provide rationale for treatment combinations including an mTOR inhibitor and temozolomide or irinotecan in certain patients.
- Both cases of KRAS mutation were accompanied by loss of PTEN, indicating this may be a potential escape mechanism to RAS/ERK inhibition.



Conclusions

✓**A low prevalence of mutations was observed in advanced / refractory TGCT with 6 mutations in 4 different genes.**

✓**TP53 mutations may be an important driver of tumorigenesis and / or a reason for treatment resistance in a subset of pts.**

✓**Biopsies taken from primary and metastatic sites were found to be comparable regarding potential targetable alterations.**

✓**Targeting the cKIT, HER2 or cMET-pathways may warrant treatment options in only a small number of individual patients.**

✓**35% of remaining patients without receptor overexpression had PTEN loss, which suggests blockade of the AKT signalling could be worth investigating further in refractory TGCT.**

✓**Alterations of the RAS/ERK pathway are rarely seen indicating that there is not an innate escape mechanism to mTOR inhibition in this tumor type.**

✓**Follow-up of treatment outcome in patients in whom targetable alterations were found is currently planned.**

References

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