

Case series

Molecular profiling of mesonephric and mesonephric-like carcinomas of cervical, endometrial and ovarian origin

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ABSTRACT

Mesonephric carcinoma is a rare cancer that most often arises within the cervix, and less frequently, in the ovary and endometrium. A retrospective search of our CLIA-certified and CAP-accredited reference molecular laboratory database (Foundation Medicine, Inc.) identified 20 mesonephric or mesonephric-like, cervical (n = 10), endometrial (n = 5), ovarian (n = 4) or peri-bladder (n = 1) carcinomas that had undergone comprehensive genomic profiling via next generation sequencing. Activating *KRAS* mutations were present in 90%, 18 of 20 cases, including G12V (n = 7), G12D (n = 6), G12A (n = 3) and G12C (n = 2). Other recurrent alterations were identified in *ARID1A* (25%), *PIK3CA* (20%), *CTNNB1* (15%), *TP53* (10%), *MLL2* (10%) and *CDKN2A* (10%). One *KRAS* wild-type case had a *GATA3* mutation as the sole alteration, while the second *KRAS* wild-type case had an *EGFR* exon 20 insertion D770_N771insSVD alteration. All tumors were negative for HPV DNA, microsatellite instability, high tumor mutational burden and homologous recombination deficiency. A circulating tumor DNA (ctDNA) liquid biopsy from peripheral blood, which was performed 6 years after original solid tumor resection in one patient with suspected lung metastasis, revealed concordance of *KRAS* alteration, gains of chromosomes 1q, 2, 10, 12 and 20, plus new *TP53* alterations in the liquid biopsy compared to the original sample. *KRAS* G12 mutation is major driver of mesonephric and mesonephric-like carcinomas, with less frequent contribution by *ARID1A* and *PIK3CA* pathways in tumors of non-cervical origin. ctDNA liquid biopsy may be useful in detecting mutations in recurrent or metastatic patients, who may potentially be eligible for trials against emerging targeted therapies.

1. Introduction

Mesonephric carcinoma is a rare cancer subtype that most often arises from mesonephric remnants within the cervix, accounting for <1% of cervical cancers (Howitt and Nucci, 2018). Patients typically present with abnormal vaginal bleeding during peri- or post-menopause (Silver et al., 2001). Due to rarity and paucity of studies with long-term follow-up, it is difficult to determine whether, stage for stage, the prognosis is different from usual-type or other variants of cervical adenocarcinoma. However, it has been suggested that mesonephric adenocarcinomas may have a propensity for late recurrence and

metastasis (Silver et al., 2001). Currently, the standard treatment for mesonephric adenocarcinomas includes a radical hysterectomy with bilateral salpingo-oophorectomy and lymph node dissection. Advanced stage tumors may also benefit from radiation and/or carboplatin plus paclitaxel-based chemotherapy (Mabuchi et al., 2009).

Recently, mesonephric-like adenocarcinomas have been described in the uterine corpus, ovary and other pelvic, periovarian or peri-uterine locations (Mirkovic et al., 2018; Kolin et al., 2019). These tumors may arise from remnants of the mesonephric duct in these locations or from differentiation of Mullerian-type carcinomas to a mesonephric cell lineage (McCluggage et al., 2020). However, these non-cervical,

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Table 1
Clinico-pathological features of mesonephric and mesonephric-like carcinomas of cervical, endometrial, ovarian and bladder/periurethral origin.

n	Age	Site of origin	Size (cm)	Sites of extension, metastasis or recurrence	Stage	Site sequenced
1	68	Cervix	8	Uterus, ovary, aortic and pelvic lymph nodes at initial hysterectomy. Subsequent recurrence as lung metastasis.	IV	Cervix
2	62	Cervix	5	Abdominal wall, peritoneum, colon, lung	IV	Colon
3	69	Bladder neck/urethra	3.8	Bladder wall, perivesical fat, vaginal wall	IV	Peri-urethra
4	66	Cervix	6.5	Lymph node recurrence	IIIC	Periaortic lymph node
5	76	Ovary	7	Uterine serosa, outer myometrium, rectum, colon	III	Ovary
6	60	Uterine corpus	3.5	Outer myometrim	IB	Uterus
7	59	Uterine corpus	6.5	Uterine serosa and ovary	III	Uterine wall
8	74	Cervix	1.4	Vaginal wall	IIIA	Vagina
9	48	Cervix	0.8	Lung-left basal lobe	IV	Lung
10	51	Cervix	2.8	Parametrium	IIB	Cervix
11	63	Cervix	0.9	Lung	IV	Lung
12	44	Cervix	5	Parametrium and ovary	IIB	Cervix
13	73	Cervix	0.8	Lung	IV	Lung
14	76	Uterine corpus	4.5	Pelvic lymph nodes	IIIC	Endometrium
15	77	Uterine corpus	2	Pelvic lymph node	IIIC	Endometrium
16	51	Cervix	1.2	Vagina, ovary, abdominal wall, colon, omentum	IV	Ovary
17	74	Pelvis/ovary	Unk.	Bladder	II	Pelvic mass
18	68	Ovary	7.5	Pelvic adnexal soft tissue	IIB	Adnexal soft tissue
19	66	Uterine corpus	12	Outer myometrim	IB	Uterus
20	69	Ovary	9.8	None	IA	Ovary

mesonephric-like tumors are exceptionally rare with endometrial mesonephric-like adenocarcinomas accounting for approximately 1% of all endometrial carcinomas (Kolin et al., 2019). In limited studies, non-cervical mesonephric-like carcinomas have been proposed to have similar morphology, overlapping immunohistochemical and genomic profiles to cervical mesonephric adenocarcinomas (Pors et al., 2018). Endometrial mesonephric-like adenocarcinomas may exhibit more aggressive behavior and higher rate of recurrence and distance metastasis, especially to lungs, compared to more commonly encountered endometrial endometrioid-type or serous-type adenocarcinomas (Euscher et al., 2019).

Recurrent *KRAS* or *NRAS* mutations have been proposed to be genomic drivers of mesonephric-type carcinomas with possible contribution by mutations in chromatin remodeling genes (i.e. *ARID1A*) (Mirkovic et al., 2015). Whether these tumors have high tumor mutational burden, microsatellite instability (biomarkers for immunotherapy), or homologous recombination deficiency (HRD; biomarker for PARP inhibitor) is unknown. In this study, our main objectives were to:

(1) analyze the genomic profiles of mesonephric-type adenocarcinomas in order to identify driver and targetable genomic alterations, (2) to further expand on the clinicopathological and genomic features of these rare tumors, including HPV status, immunotherapy biomarkers and HRD status.

2. Case series

Here, we retrospectively identify 20 mesonephric or mesonephric-like carcinomas, which had previously undergone comprehensive genomic profiling, from the archives of a large CLIA-certified and CAP-accredited reference molecular laboratory (Foundation Medicine, Inc.). Of the 20 cases, 10 were of cervical origin; 5 originated in the uterine corpus/endometrium; 4 were of ovarian or pelvic origin and 1 case originated in the bladder/peri-urethral region. In this cohort of 20 cases, patient age ranged from 44 to 77 years with a median age of 67 years (Table 1). Primary tumor size ranged from 1.2 to 12 cm with a median size of 4.8 cm. Most tumors in our study were high stage and aggressive with spread of tumor beyond the uterus. Specifically, 15% of cases were stage I, 20% stage II, 30% stage III, and 35% stage IV (Table 1). In addition, a small subset of tumors (4 of 20) exhibited distant metastasis to lung (Table 1).

Morphologically, the tumors exhibited various architectural patterns, including tubulo-papillary, solid and spindle, corded and nested and glandular with luminal secretions, compatible with mesonephric-type adenocarcinoma (Fig. 1). Cervical tumors invaded deeply into cervical wall with classic mesonephric morphology (Fig. 1A and B). Ovarian (Fig. 1C and D), endometrial (Fig. 1E and F) and bladder/periurethral mesonephric-type adenocarcinomas exhibited similar morphology to their cervical counterparts. In addition, for the bladder/periurethral case, benign mesonephric remnants were identified in the peri-urethral location, consistent with mesonephric origin.

Comprehensive genomic profiling via targeted next-generation sequencing of up to 324 genes involved in tumorigenesis was performed at Foundation Medicine, Inc. as previously described (Lin et al., 2017) and revealed activating *KRAS* missense mutations at position G12 in the majority, 90%, 18 of 20 cases (Fig. 2). All *KRAS* mutations occurred at position G12 and included: G12V (n = 7), G12D (n = 6), G12A (n = 3) and targetable G12C (n = 2). Other recurrent oncogenic alterations were identified in *ARID1A* (25%, 5 of 20), *PIK3CA* (20%, 4 of 20), *CTNBN1* (15%, 3 of 20), *TP53* (10%, 2 of 20), *MLL2* (10%, 2 of 20), *CDKN2A* (10%, 2 of 20) and *BCOR* (10%, 2 of 20) (Fig. 2). One *KRAS* wild-type cervical case had a *GATA3* R262fs*42, frameshift mutation as the sole driver alteration, while the second *KRAS* wild-type case, which was of endometrial origin, had an activating *EGFR* exon 20 insertion D770_N771insSVD (Fig. 2).

Examination of copy number plots demonstrated gain of chromosome 1q in 95%, 19 of 20 cases. Additional, high frequency and recurrent chromosome gains included chromosome 10 at 60% (12 of 20) of cases and chromosome 20 at 60% (12 of 20) of cases. Frequent chromosome losses included chromosome 1p at 45% (9 of 20), chromosome 22 at 30% (6 of 20), chromosomes 9 at 25% (5 of 20) and chromosome 18 at 25% (5 of 20) of cases.

In our cohort, *ARID1A* and *PIK3CA* genomic alterations were identified only in mesonephric-like carcinomas of endometrial or ovarian origin compared to mesonephric carcinomas of cervical origin. Furthermore, gain of chromosome 12 was identified only in carcinomas of cervical or ovarian origin but not in carcinomas of endometrial origin.

Composite biomarker analysis revealed that none of the tumors had a high mutation burden (mean 3.7 mut/Mb; high TMB defined as >19mut/Mb) and were not microsatellite unstable, which are two established immunotherapy biomarkers. In addition, using the validated methods from our CLIA-certified laboratory for PARP inhibitor

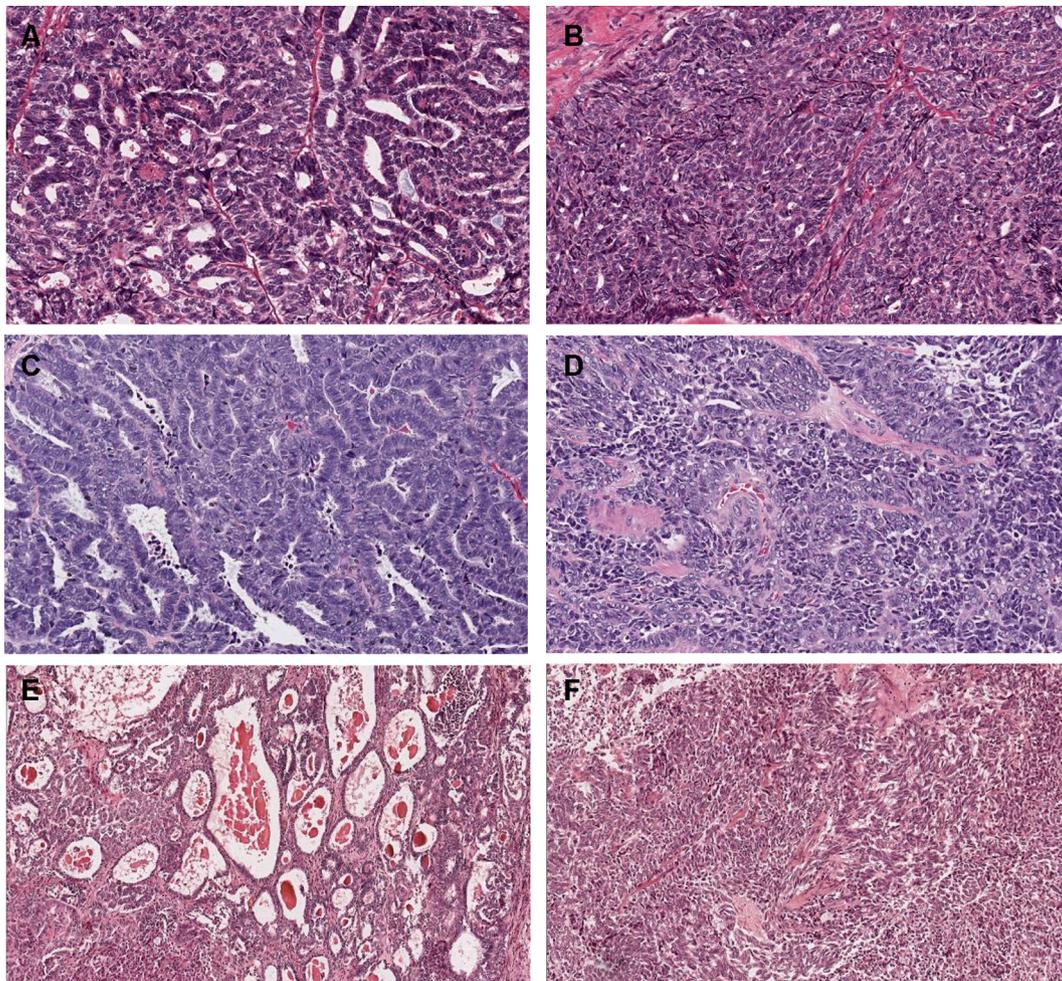


Fig. 1. Representative cases of cervical, ovarian and endometrial mesonephric and mesonephric-like carcinomas. Cervical mesonephric adenocarcinoma corresponding to case #1 with (A) tubular and glandular architecture with luminal secretions and (B) solid and spindle cell morphology. (C–D): Ovarian mesonephric-like adenocarcinoma corresponding to case #18 with (C) tubulo-glandular architecture and (D) solid and spindle cell morphology. Representative endometrial mesonephric-like adenocarcinoma corresponding to case #19 with (E) tubulo-cystic and glandular architecture with luminal secretions and (F) solid and spindle cell morphology.

effectiveness in the ARIEL3 study for ovarian cancer (NCT01968213) (Coleman et al., 2017), none of the tumors had high genome-wide loss of heterozygosity scores (high gLOH defined as >16% for ovarian cancer), which is a biomarker for HRD and PARP inhibitor therapy. Finally, all 20 tumors were negative for viral low risk HPV 6 and 11 and for high risk HPV 16 and 18 DNA by HPV-specific next generation sequencing.

In one patient, a peripheral blood liquid biopsy was obtained to guide care six years after solid tumor testing, which revealed concordance of high level *KRAS* G12D alteration with the prior sample as well as new *TP53* alterations, R249S and R280G, in the liquid biopsy compared to the prior solid tumor sample (Fig. 3B). In addition, both solid and liquid biopsy specimens had concordant gains of chromosome 1q, 2, 10, 12 and 20, supporting the clinical impression of metastatic cervical mesonephric carcinoma to lung (Fig. 3C).

3. Discussion

In this study, we expand on the genomic and clinicopathological characteristics of mesonephric and mesonephric-like carcinomas of cervical, endometrial and ovarian origin. Patient age in our cohort ranged from 42 to 70 years, with a median age of 67 years, similar to

prior studies (Mirkovic et al., 2018, 2015). We validate *KRAS* G12 alterations, as well as high frequency of gains of chromosomes 1q, 10, 12, 20 and loss of chromosome 1p in these tumors. Similar to cervical tumors, mesonephric-like carcinomas of ovarian or endometrial origin had overlapping morphological features and were driven by *KRAS* G12 alterations with recurrent chromosomal gains and losses. Similar to prior reports (Mirkovic et al., 2018), co-alterations in *PIK3CA* and *ARID1A* specifically occurred in mesonephric-like carcinomas of non-cervical origin, arising either in the endometrial uterine corpus or ovary, suggesting the possibility that ovarian or endometrial mesonephric-like carcinomas may be endometrioid-type tumors that underwent mesonephric-like differentiation.

One *KRAS* wild-type cervical mesonephric carcinoma was driven only by a *GATA3* mutation, which is a transcription factor and marker of mesonephric lineage differentiation (Howitt et al., 2015). Alterations in lineage-specific transcription factors with associated tumor addiction have been described in other tumor types, such as alterations of Mullerian-specific *PAX8* and associated tumor dependency on *PAX8* in ovarian cancer (Cheung et al., 2011). In contrast, the only other *KRAS* wild-type case was an endometrial mesonephric-like carcinoma, which had co-alterations in *EGFR*, *PIK3CA*, *ARID1A*, *TP53*, *CDKN2A* and

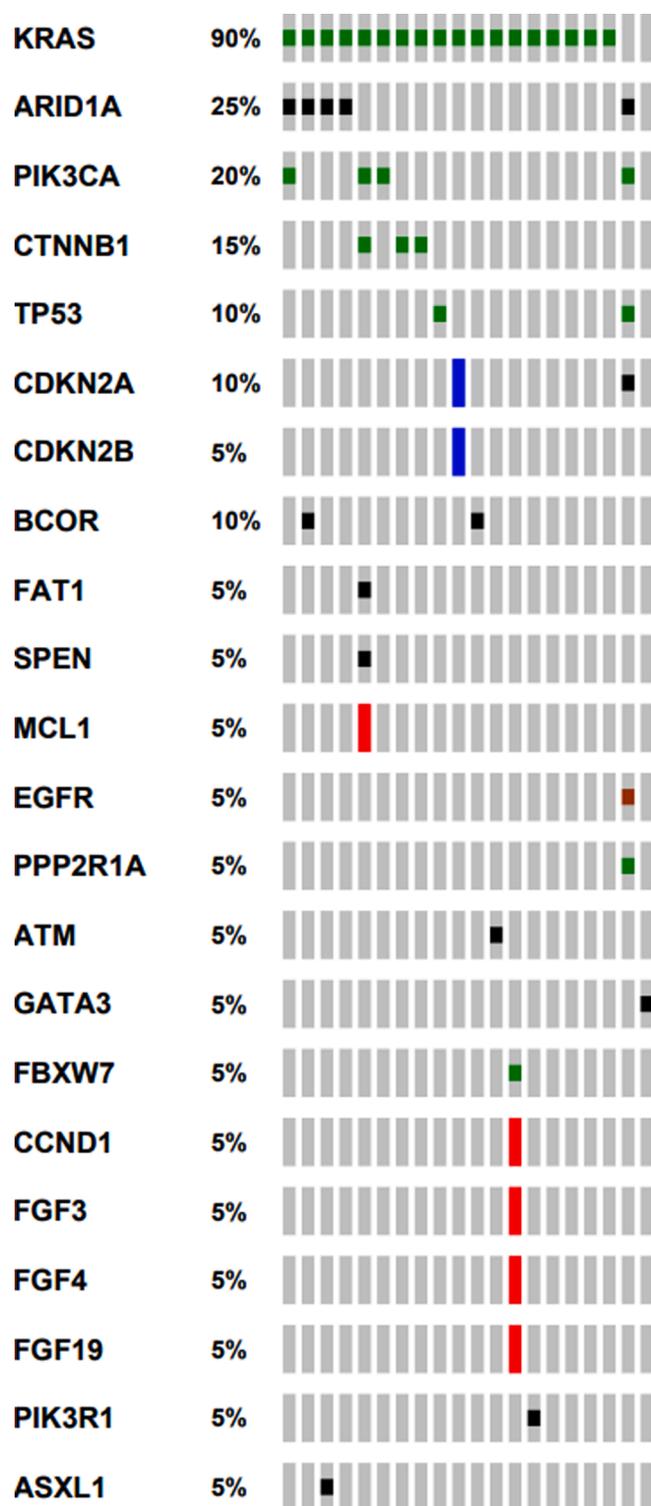


Fig. 2. Mutational landscape and genomic profiles of *KRAS* across mesonephric and mesonephric-like carcinomas. Each column represents one tumor. Squares = short variant alterations. Green = Missense driver mutation. Black = truncation. Red rectangle = amplification. Blue rectangle = homozygous deletion. Variants of unknown significance were excluded.

PPP2R1A. This tumor lacked typical chromosomal gains or losses of 1q, 10, 12, 20, suggesting that this may also have been a high-grade endometrioid-type tumor that underwent mesonephric-like differentiation.

Our results reveal opportunities for personalized medicine and

targeted therapies in mesonephric-type carcinomas. Given that activating *KRAS* alterations are the major oncogenic drivers regardless of specific site of origin, these tumors may be sensitive to MEK inhibition, which is downstream of *KRAS*, such as with trametinib and cobimetinib. More recently, the specific short variant *KRAS* mutation, *KRAS* G12C, has emerged as an anticancer target with two novel *KRAS* G12C-specific kinase inhibitors (Amgen AMG510; Miratti MRTX849), showing significant efficacy in a variety of cancer types in early studies (Caruso, 2019). Identification of *KRAS* G12C in our cohort suggests that mesonephric-type carcinomas may be susceptible to these novel anti-*KRAS* G12C-specific agents, which are currently in clinical trials (i.e. NCT03600883 and NCT03785249).

Less frequently and specifically in endometrial and ovarian mesonephric-like carcinomas and not in cases of cervical origin, activating *PIK3CA* mutations were identified in a subset of the tumors. Based on evidence in other *PIK3CA*-mutated tumor types, this subset of *PIK3CA*-mutated mesonephric-like tumors may respond to mTOR inhibitors. More recently, a *PIK3CA*-specific inhibitor, Alpelisib, has been FDA-approved for *PIK3CA*-mutated, hormone receptor-positive, advanced breast cancers, suggesting that Alpelisib may be effective in a subset of *PIK3CA*-mutated mesonephric-like carcinomas. Interestingly, one case of mesonephric-like endometrial adenocarcinoma in our cohort exhibited double *PIK3CA* E453K and Q546H mutations, and double *PIK3CA* mutations have previously shown to increase *PIK3CA* oncogenicity and sensitivity to PI3K inhibition in other systems (Vasan et al., 2019). However, we do not have any data on treatment and response of any patient with regards to any targeted therapy. This study limitation may be an opportunity for future investigation.

In conclusion, genomic profiling of mesonephric-type carcinomas via solid tumor and liquid biopsy demonstrates that activating alterations in *KRAS*, including targetable G12C, is a major driver event in mesonephric and mesonephric-like carcinomas, with less frequent contribution by *ARID1A* and *PIK3CA* pathways in tumors of ovarian or endometrial origin. Our results reveal potential benefit from targeted therapies against *KRAS*/MEK and PI3K/mTOR pathways in mesonephric-type carcinoma of the gynecological tract and the utility of liquid biopsy in detecting targetable mutations in the recurrence or metastasis of this family of tumors.

CRediT authorship contribution statement

Douglas I. Lin: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Nikunj Shah:** Data curation, Formal analysis. **Julie Y. Tse:** Formal analysis, Writing - original draft, Writing - review & editing. **Jonathan K. Killian:** Formal analysis. **Amanda Hemmerich:** Formal analysis, Writing - original draft, Writing - review & editing. **Claire Edgerly:** Formal analysis, Writing - original draft, Writing - review & editing. **James Haberberger:** Formal analysis. **Eric A. Severson:** Formal analysis, Writing - original draft, Writing - review & editing. **Richard S.P. Huang:** Formal analysis, Writing - original draft, Writing - review & editing. **Shakti H. Ramkissoon:** Writing - original draft, Writing - review & editing. **Jo-Anne Vergilio:** Project administration. **Jeffrey S. Ross:** Formal analysis, Project administration, Writing - original draft, Writing - review & editing. **Julia A. Elvin:** Conceptualization, Project administration.

Declaration of Competing Interest

The authors declare that they are full-time employees of Foundation Medicine Inc., which is a whole subsidiary of Roche.

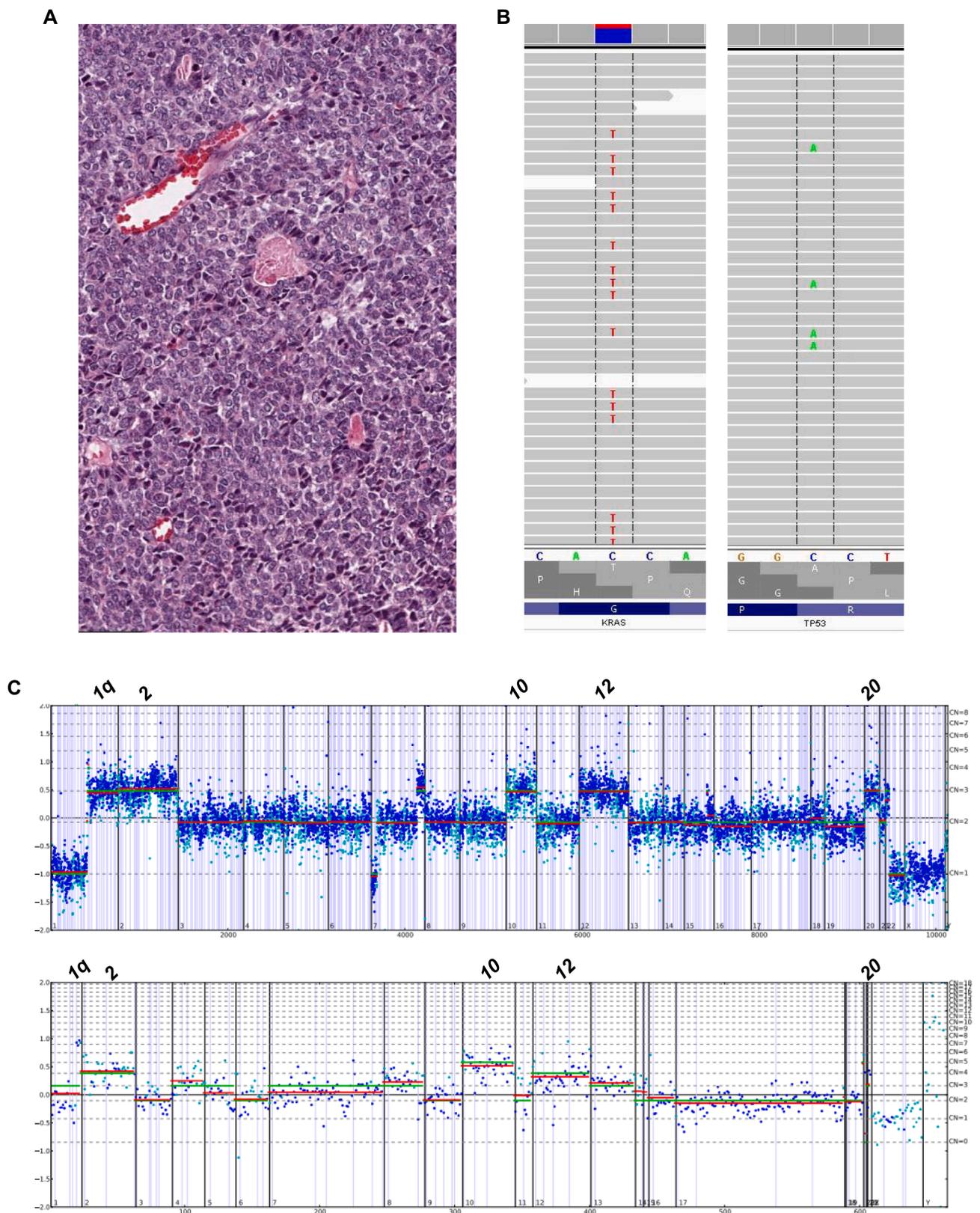


Fig. 3. (A) H&E of cervical mesonephric carcinoma corresponding to case #2 with *KRAS* G12D as the only solid tumor genomic alteration. (B) Next-generation sequencing results of cell free circulating tumor DNA liquid biopsy from a peripheral blood sample from the same patient 6 years later exhibiting the same *KRAS* alteration (left) and additional *TP53* alteration (right) at a lower frequency. (C) Genome wide copy number plots of solid (top) and liquid biopsy (bottom) demonstrating gains of chromosome 1q, 2, 10, 12 and 20 in both solid tumor and liquid biopsy samples.

References

- Caruso, Catherine, 2019. AMG 510 First to inhibit “Undruggable” KRAS. *Cancer Discov.* 9 (8) <https://doi.org/10.1158/2159-8290.CD-NB2019-073>, 988.3–989.
- Cheung, H.W., Cowley, G.S., Weir, B.A., et al., 2011. Systematic investigation of genetic vulnerabilities across cancer cell lines reveals lineage-specific dependencies in ovarian cancer. *Proc. Natl. Acad. Sci. U. S. A.* 108 (30), 12372–12377.
- Coleman, R.L., Oza, A.M., Lorusso, D., et al., 2017. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 390 (10106), 1949–1961.
- Euscher, E.D., Bassett, R., Duose, D.Y., et al., 2019. Mesonephric-like carcinoma of the endometrium. *Am. J. Surg. Pathol.* 1 <https://doi.org/10.1097/pas.0000000000001401>.
- Howitt, B.E., Emori, M.M., Drapkin, R., et al., 2015. GATA3 Is a sensitive and specific marker of benign and malignant mesonephric lesions in the lower female genital tract. *Am. J. Surg. Pathol.* 39 (10), 1411–1419.
- Howitt, B.E., Nucci, M.R., 2018. Mesonephric proliferations of the female genital tract. *Pathology* 50 (2), 141–150.
- Kolin, D.L., Costigan, D.C., Dong, F., Nucci, M.R., Howitt, B.E., 2019. A combined morphologic and molecular approach to retrospectively identify KRAS-mutated mesonephric-like adenocarcinomas of the endometrium. *Am. J. Surg. Pathol.* 43 (3), 389–398.
- Lin, D.I., Chudnovsky, Y., Duggan, B., et al., 2017. Comprehensive genomic profiling reveals inactivating SMARCA4 mutations and low tumor mutational burden in small cell carcinoma of the ovary, hypercalcemic-type. *Gynecol. Oncol.* 147 (3), 626–633. <https://doi.org/10.1016/j.ygyno.2017.09.031>, 29102090.
- Mabuchi, S., Morishige, K., Fujita, M., et al., 2009. The activity of carboplatin and paclitaxel for recurrent cervical cancer after definitive radiotherapy. *Gynecol. Oncol.* 113 (2), 200–204.
- Mirkovic, J., Sholl, L.M., Garcia, E., et al., 2015. Targeted genomic profiling reveals recurrent KRAS mutations and gain of chromosome 1q in mesonephric carcinomas of the female genital tract. *Mod. Pathol.* 28 (11), 1504–1514.
- McCluggage, W.G., Vosmikova, H., Laco, J., 2020. Ovarian combined low-grade serous and mesonephric-like adenocarcinoma: Further Evidence for A Mullerian Origin of Mesonephric-like Adenocarcinoma. *Int. J. Gynecol. Pathol.* 1, 84–92. <https://doi.org/10.1097/PGP.0000000000000573>, 30575604.
- Mirkovic, J., McFarland, M., Garcia, E., et al., 2018. Targeted genomic profiling reveals recurrent KRAS mutations in mesonephric-like adenocarcinomas of the female genital tract. *Am. J. Surg. Pathol.* 42 (2), 227–233.
- Pors, J., Cheng, A., Leo, J.M., Kinloch, M.A., Gilks, B., Hoang, L., 2018. A comparison of GATA3, TTF1, CD10, and calretinin in identifying mesonephric and mesonephric-like carcinomas of the gynecologic tract. *Am. J. Surg. Pathol.* 42 (12), 1596–1606.
- Silver, S.A., Devouassoux-Shisheboran, M., Mezzetti, T.P., Tavassoli, F.A., 2001. Mesonephric adenocarcinomas of the uterine cervix: a study of 11 cases with immunohistochemical findings. *Am. J. Surg. Pathol.* 25 (3), 379–387.
- Vasan, N., Razavi, P., Johnson, J.L., et al., 2019. Double PIK3CA mutations in cis increase oncogenicity and sensitivity to PI3Ka inhibitors. *Science* 366 (6466), 714–723.