The Mutational Profile of Unicystic Ameloblastoma

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Appendix

Appendix Table 1. Clinico-pathological information and mutation status of the unicystic ameloblastomas (N=39).

UAM	Clinicopathological data								Mutation statuts		
Case	Age	Sex	Ethnicity	Location	Unerupted tooth	Primary Rec	Treatment	Histological type	BRAF V600E Genotyping	BRAF V600E IHC	NGS
1	85	M	Caucasian	Mandible	NO	Primary	Enucleation	Luminal	V600E	Positive	N.A.
2	60	M	Inuit	Mandible	NA	Primary	Enucleation	Mural	V600E	Positive	N.A.
3	42	M	Caucasian	Maxilla	YES	1 Rec	Enucleation	Intraluminal	DNW	Positive	N.A.
4	24	M	Asian	Mandible	YES	Primary	Enucleation	Mural	V600E	Positive	N.A.
5	27	F	Caucasian	Mandible	YES	Primary	Enucleation	Luminal	DNW	Negative	N.A.
6	28	M	Asian	Mandible	YES	Primary	Enucleation	Mural	V600E	Positive	N.A.
7	10	M	Caucasian	Mandible	YES	Primary	Enucleation	Luminal	DNW	Negative	N.A.
8	31	M	Asian	Mandible	YES	Primary	Enucleation	Luminal	V600E	Positive	N.A.
9	20	M	Afro Caribbean	Maxilla	NO	Primary	Enucleation	Luminal	V600E	Positive	N.A.
10	47	F	Caucasian	Mandible	NO	Primary	Enucleation	Luminal	DNW	Positive	N.A.
11	15	F	Caucasian	Mandible	YES	Primary	Enucleation	Luminal	DNW	Negative	N.A.
12	14	M	Caucasian	Maxilla	YES	1 Rec	Enucleation	Luminal	WT	Negative	Negative
13	7	F	Afro Caribbean	Maxilla	NO	Primary	Enucleation	Luminal	WT	Negative	Negative
14	18	F	Caucasian	Mandible	YES	Primary	Enucleation	Mural	V600E	Positive	N.A.
15	14	F	Afro Caribbean	Mandible	YES	Primary	Enucleation	Mural	V600E	Positive	N.A.
16	65	F	Caucasian	Mandible	NO	Primary	Enucleation	Luminal	V600E	Positive	N.A.
17	50	M	Caucasian	Mandible	YES	Primary	Resection	Intraluminal	WT	Negative	SMO p.L412F
18	17	F	Caucasian	Mandible	YES	Primary	Enucleation	Mural	V600E	Positive	N.A.
19	17	F	Caucasian	Mandible	NO	Primary	Enucleation	Mural	V600E	Positive	N.A.
20	25	F	Caucasian	Mandible	YES	Primary	Enucleation	Mural	V600E	Positive	N.A.
21	24	F	N.A.	Mandible	NO	Primary	Resection	Luminal	V600E	Positive	N.A.
22	64	F	Caucasian	Mandible	NO	2 Rec	Enucleation	Intraluminal	V600E	Positive	N.A.
23	68	F	Caucasian	Mandible	NO	3 Rec	Enucleation	Intraluminal	V600E	Positive	N.A.
24	66	F	Caucasian	Mandible	NO	Primary	Enucleation	Mural	V600E	Positive	N.A.
25	34	M	Caucasian	Mandible	NO	1 Rec	Resection	Mural	V600E	Positive	N.A.
26	23	F	Caucasian	Mandible	YES	1 Rec	Enucleation	Mural	V600E	Positive	N.A.
27	22	F	Caucasian	Mandible	YES	Primary	Enucleation	Intraluminal	V600E	Positive	N.A.
28	17	F	Caucasian	Mandible	YES	Primary	Resection	Luminal	V600E	Positive	N.A.
29	64	M	Caucasian	Mandible	NO	Primary	Enucleation	Intraluminal	WT	Negative	Negative
30	35	F	Caucasian	Mandible	NO	1 Rec	Enucleation	Mural	V600E	Positive	N.A.
31	25	M	Caucasian	Mandible	YES	Primary	Enucleation	Mural	V600E	Positive	N.A.
32	63	F	Caucasian	Mandible	NO	Primary	Resection	Luminal	V600E	Positive	N.A.
33	16	M	Caucasian	Mandible	YES	Primary	Enucleation	Intraluminal	V600E	Positive	N.A.
34	55	M	Caucasian	Mandible	YES	Primary	Enucleation	Intraluminal	V600E	Positive	N.A.
35	29	F	Caucasian	Mandible	YES	3 Rec	Enucleation	Mural	V600E	Positive	N.A.
36	19	F	Caucasian	Mandible	YES	Primary	Enucleation	Mural	V600E	Positive	N.A.
37	15	M	Caucasian	Mandible	YES	Primary	Enucleation	Luminal	V600E	Positive	N.A.
38	15	M	Caucasian	Mandible	YES	Primary	Enucleation	Intraluminal	V600E	Positive	N.A.
39	15	M	N.A.	Mandible	YES	1 Rec	Enucleation	Luminal	V600E	N.A.	N.A.

Abbreviations: F female; M male; NA not available, N.A. not analyzed; WT wild type; G genotyping; S Sanger sequencing; NGS next generation sequencing; Rec recurrence.

Appendix Table 2. Clinico-pathological information and mutation status of the conventional ameloblastomas (N=39).

AM	Clinicopathological data							Mutation statuts							
Case	Age	Sex	Ethnicity	Location	Primary Rec	Treatment	Histological type	BRAF status	Geno-typing Sanger	BRAF V600E IHC	KRAS status Sanger	NRAS status Sanger	HRAS status Sanger	NRAS/ HRAS IHC	NGS
1	66	M	Caucasian	Mandible	Primary	Enucleation	Follicular	V600E	S	Positive	WT	WT	WT	Negative	N.A.
2	70	F	Asian	Mandible	Primary	Enucleation	Follicular	V600E	S	Positive	WT	WT	WT	Negative	N.A.
3	61	M	Caucasian	Mandible	1 Rec	Resection	Plexiform	WT	S	Negative	WT	WT	WT	Negative	FGFR2 p.C382R
4	27	F	Black Somali	Mandible	Primary	Enucleation	Follicular	V600E	S	Positive	WT	WT	WT	Negative	N.A.
5	24	F	Black	Mandible	Primary	Enucleation	Plexiform	V600E	S	Positive	WT	WT	WT	Negative	N.A.
6	50	F	Black	Mandible	Primary	Resection	Plexiform	V600E	S	Negative	WT	WT	WT	Negative	N.A.
7	36	M	Afro Caribbean	Mandible	Primary	Enucleation	Follicular	WT	S	Negative	WT	Q61R	WT	Positive	N.A.
8	47	M	Caucasian	Mandible	Primary	Resection	Follicular	V600E	S	Positive	WT	WT	WT	Negative	N.A.
9	32	M	North African	Mandible	1 Rec	Enucleation	Plexiform	WT	S	N.A.	WT	WT	WT	Negative	N.A.
10	46	M	African	Mandible	2 Rec	Resection	Follicular	V600E	S	N.A.	WT	WT	WT	Negative	N.A.
11	14	M	Black North African	Mandible	Primary	Enucleation	Plexiform	V600E	S	Positive	WT	WT	WT	Negative	N.A.
12	34	F	Caucasian	Mandible	1 Rec	Resection	Follicular	V600E	S	Positive	WT	WT	WT	Negative	N.A.
13	84	M	Caucasian	Mandible	Primary	Resection	Follicular	WT	GS	Negative	WT	WT	WT	Negative	Negative
14	18	F	Caucasian	Mandible	Primary	Enucleation	Follicular	V600E	S	N.A.	WT	WT	WT	Negative	N.A.
15	16	M	Caucasian	Mandible	Primary	Enucleation	Plexiform	V600E	S	Positive	WT	WT	WT	Negative	N.A.
16	61	F	N.A.	Mandible	Primary	Enucleation	Plexiform follicular	WT	S	Negative	WT	WT	WT	Negative	Negative
17	77	M	Caucasian	Mandible	1 Rec	Resection	Plexiform	WT	S	Negative	WT	WT	Q61R	Positive	N.A.
18	69	M	Caucasian	Mandible	Primary	Resection	Plexiform	WT	S	Negative	WT	WT	Q61R	Positive	N.A.
19	43	M	Caucasian	Mandible	Primary	Enucleation	Follicular	V600E	S	Positive	WT	WT	WT	Negative	N.A.
20	44	F	Black African	Mandible	Primary	Enucleation, Carnoy's	Plexiform follicular	V600E	S	Positive	WT	WT	WT	Negative	N.A.
21	33	M	African	Mandible	2 Rec	Enucleation	Follicular	V600E	S	Positive	WT	WT	WT	Negative	N.A.
22	46	F	Afro Caribbean	Mandible	1 Rec	Resection	Plexiform	V600E	S	N.A.	WT	WT	WT	Negative	N.A.
23	62	M	Asian	Mandible	Primary	Enucleation	NA	V600E	G	Positive	N.A.	N.A.	N.A.	Negative	N.A.
24	31	M	Black African	Mandible	1 Rec	Enucleation	Follicular	WT	S	Negative	WT	Q61R	WT	Positive	N.A.
25	34	F	Caucasian	Mandible	1 Rec	Resection	Follicular	V600E	G	Positive	N.A.	N.A.	N.A.	Negative	N.A.
26	11	M	Caucasian	Mandible	Primary	Resection	Follicular	WT	G	Negative	WT	WT	WT	Negative	Negative
27	35	M	Caucasian	Mandible	1 Rec	Enucleation, Carnoy's	Follicular	V600E	G	Positive	N.A.	N.A.	N.A.	Negative	N.A.
28	52	M	Caucasian	Mandible	Primary	Enucleation	Follicular	V600E	G	N.A.	N.A.	N.A.	N.A.	Negative	N.A.
29	78	M	Caucasian	Mandible	Primary	Enucleation	Follicular	V600E	G	N.A.	N.A.	N.A.	N.A.	Negative	N.A.
30	39	M	N.A.	Mandible	1 Rec	Enucleation	Follicular	V600E	G	N.A.	N.A.	N.A.	N.A.	Negative	N.A.
31	36	F	Caucasian	Mandible	2 Rec	Resection	Follicular	V600E	G	Positive	N.A.	N.A.	N.A.	Negative	N.A.
32	23	F	Caucasian	Mandible	1 Res	Enucleation	Follicular	V600E	G	Positive	N.A.	N.A.	N.A.	Negative	N.A.
33	37	F	Caucasian	Mandible	1 Rec	Resection	Follicular granular cell	V600E	G	Positive	N.A.	N.A.	N.A.	Negative	N.A.
34	34	F	Caucasian	Mandible	Primary	Enucleation	Plexiform	V600E	G	Negative	N.A.	N.A.	N.A.	Negative	N.A.
35	74	F	Caucasian	Maxilla	2 Rec	Resection	Plexiform	WT	G	Negative	N.A.	N.A.	N.A.	Negative	Negative
36	71	F	Caucasian	Mandible	2 Rec	Resection	Acanthomatous	V600E	G	Negative	N.A.	N.A.	N.A.	Negative	N.A.
37	24	M	Caucasian	Mandible	Primary	Enucleation	Plexiform	V600E	G	Positive	N.A.	N.A.	N.A.	Negative	N.A.
38	53	F	Caucasian	Mandible	1 Rec	Enucleation	Plexiform	V600E	G	Positive	N.A.	N.A.	N.A.	Negative	N.A.
39	17	F	Caucasian	Mandible	1 Rec	Resection	Plexiform	WT	S	N.A.	N.A.	N.A.	N.A.	N.A.	FGFR2 p.C382R

Abbreviations: F female; M male; NA not available; N.A. not analyzed; WT wild type; G genotyping; S Sanger sequencing; NGS next generation sequencing; Rec recurrence.

Appendix Table 3. Follow-up information of the unicystic ameloblastoma patients (N=39).

	Follow up in months						
Case	Age at primary detection	1st recurrence time after	2nd recurrence time after	3rd recurrence time after	4th recurrence time after	Follow up from primary detection	Recurrence at the end of the follow-
1	85					48	NO
2	60					25	NO
3	41	12				192	NO
4	24						
5	27						
6	28					72	NO
7	10						
8	31						
9	20					60	NO
10	47						
11	15						
12	12	24				84	NO
13	7					96	NO
14	18					24	NO
15	14					60	NO
16	65					84	NO
17	50					10	NO
18	17					7	NO
19	17					16	NO
20	25					17	NO
21	24					24	NO

22	NA	N.A.	N.A.			46	NO
23	NA			46		92	YES
24	66					47	NO
25	12	265				337	NO
26	19	46	90			123	NO
27	22					79	YES
28	17					99	NO
29	64					99	NO
30	33	19				121	NO
31	25					106	NO
32	63					130	NO
33	16					153	NO
34	55	44				170	NO
35	20	16	79	103	199	280	NO
36	19					177	YES
37	15	10				180	NO
38	15					182	YES
39	13	19				19	YES

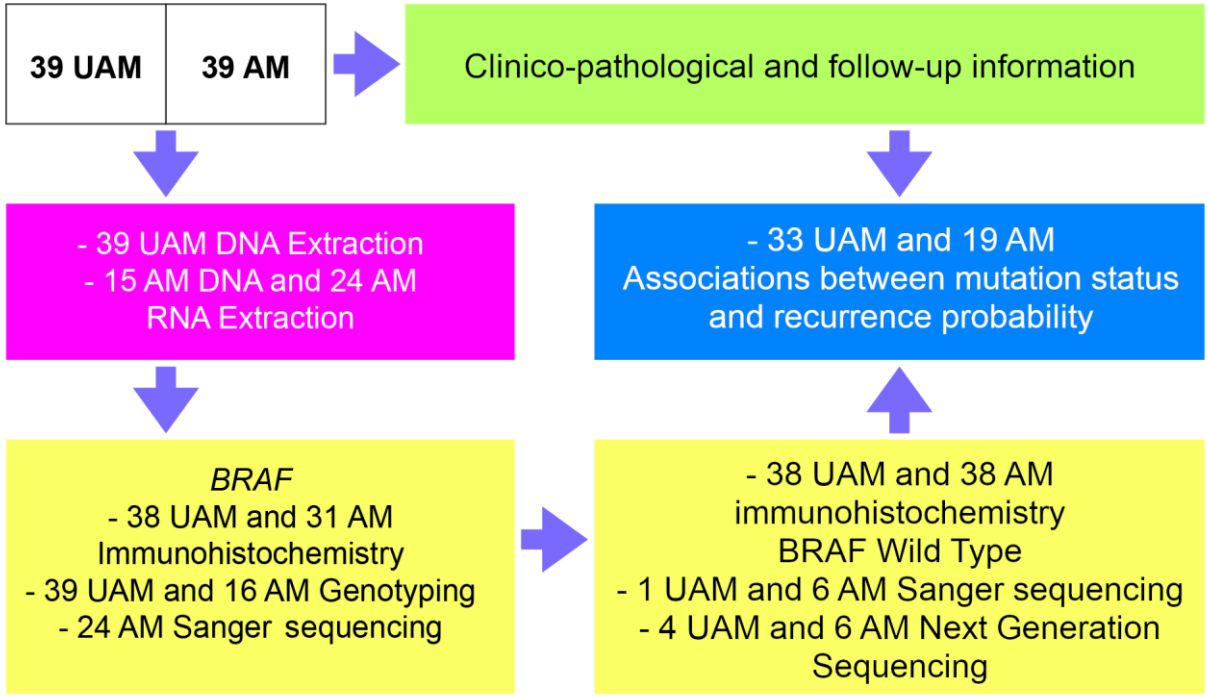
Abbreviations: NA not available.

Appendix Table 4. Follow-up information of the conventional ameloblastoma patients (N=39).

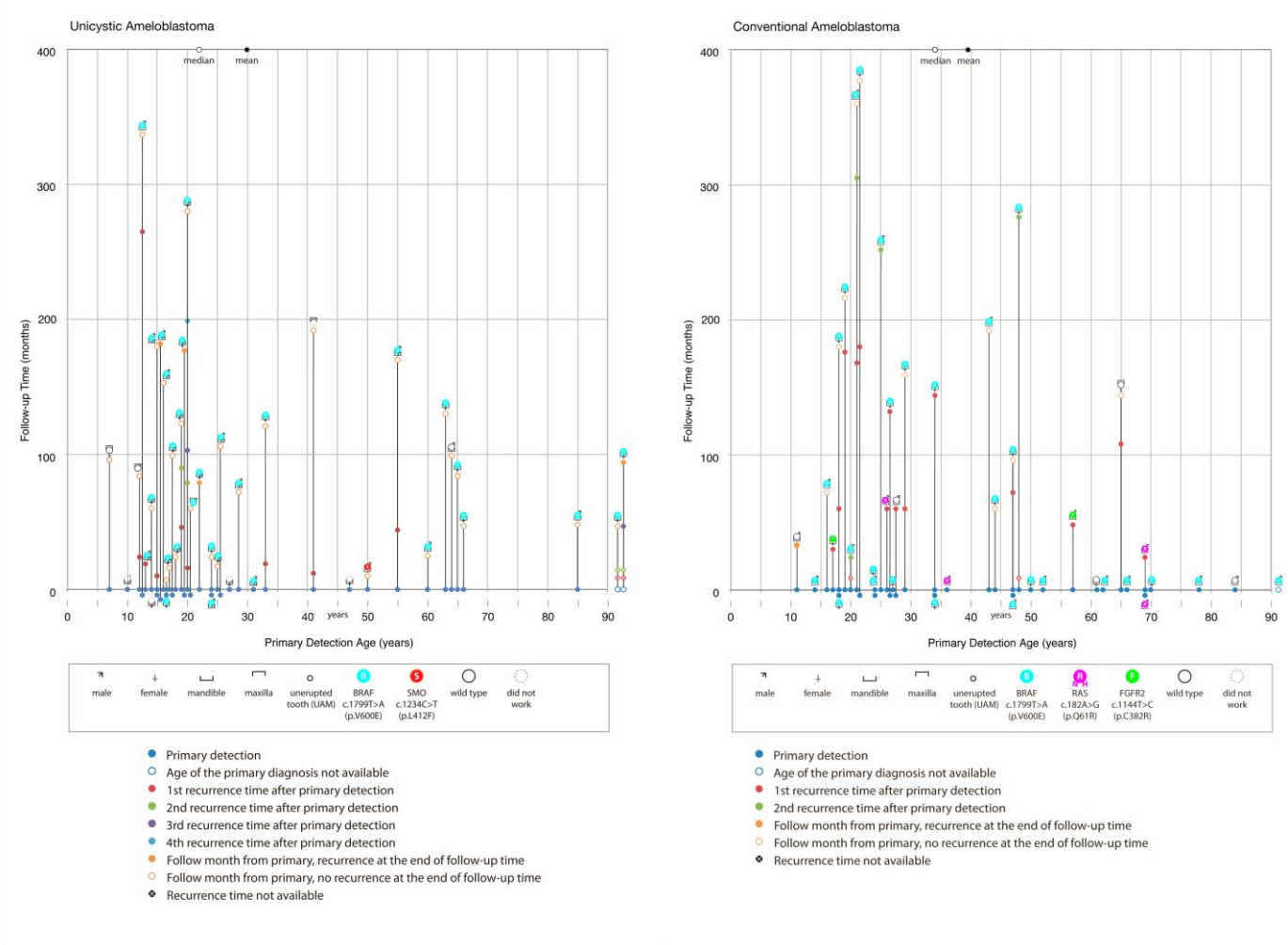
	Follow up in months				
Case	Age at primary detection (years)	1st recurrence time after primary detection	2nd recurrence time after primary	Follow up from primary detection	Recurrence at the end of the follow- up period
1	66				
2	70				
3	57	48		48	YES
4	27				
5	24				
6	50				
7	36				
8	47				
9	27	60		60	YES
10	25	NA	252	252	YES
11	14				
12	19	176		216	NO
13	84				
14	18				
15	16			72	NO
16	61				
17	69	24		24	YES
18	69				
19	43			192	NO
20	44			60	NO
21	20	NA	24	24	YES

22	34	144		144	YES
23	62				
24	26	60		60	YES
25	29	60		159	NO
26	11			33	NO
27	21	168		360	NO
28	52				
29	78				
30	NA				
31	21	180	305	377	NO
32	18	60		180	NO
33	26	132		132	YES
34	34				
35	65	108		144	NO
36	48	NA	276	276	YES
37	24				
38	47	72		96	NO
39	17	30		30	YES

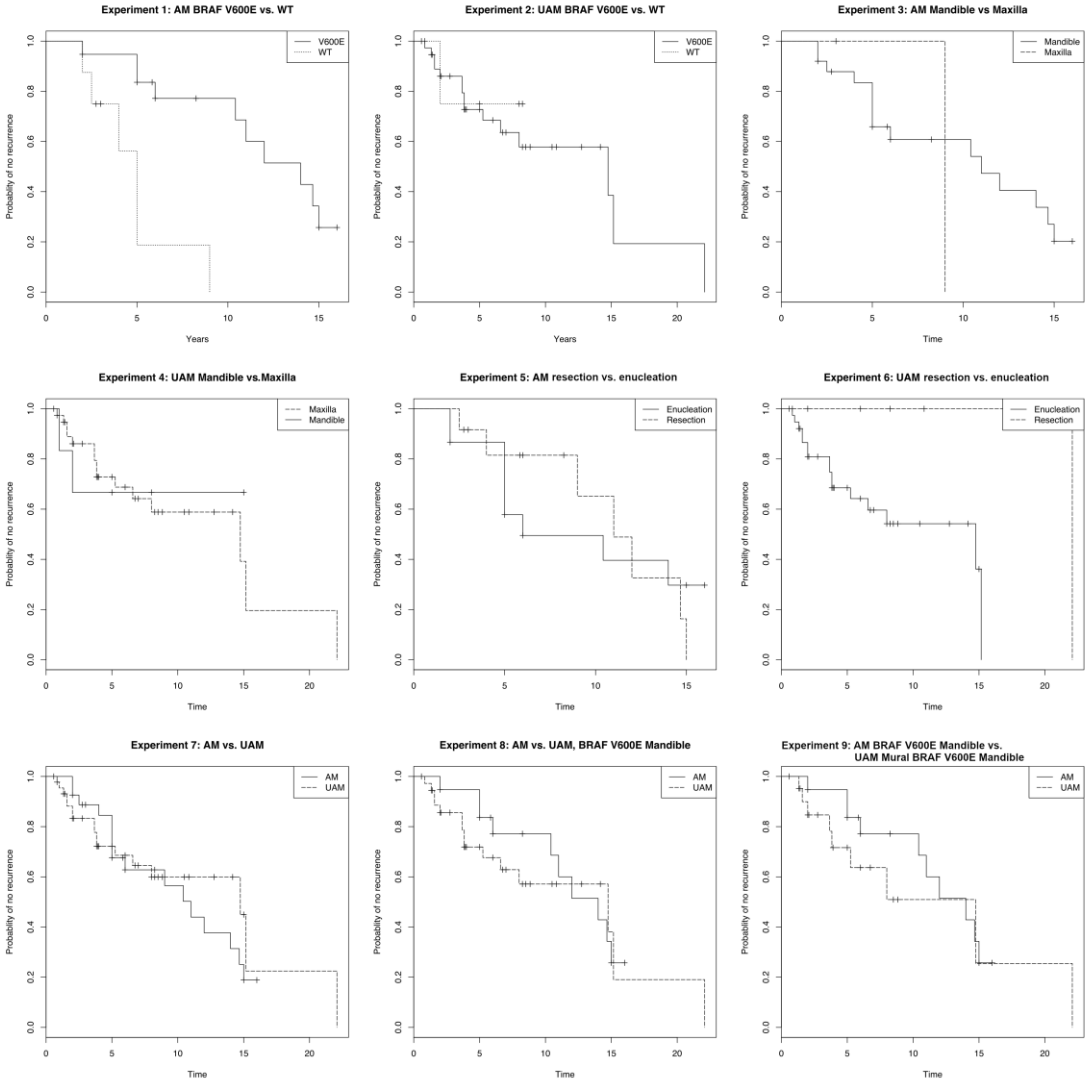
Abbreviations: NA not available



Appendix Figure 1. Flow chart of the study. Clinico-pathological and follow-up information were collected from 39 UAM and 39 AM patients. DNA was extracted from 39 UAM and 15 AM FFPE blocks and RNA from 24 fresh frozen AM tissues. *BRAF* V600E mutations were first analyzed by VE1 immunohistochemistry (IHC) from 38 UAMs and 31AMs, by genotyping from 39 UAMs and 16 AMs and by Sanger sequencing from 24 AMs. Ameloblastomas where thereafter analyzed by RAS p.Q61R IHC. Four AMs were RAS IHC positive. The presence of RAS mutations in these four AMs were confirmed by Sanger sequencing. Two BRAF wild-type UAMs underwent targeted next-generation sequencing (NGS). NGS was also performed for four wild-type UAMs and six wild-type AMs. One UAM harbored SMO p.L412F mutation and two AMs FGFR2 p.C382R mutations, which were confirmed by Sanger sequencing. Finally, Kaplan-Meier-analyses were performed on 33 UAMs and 19 AMs, which had follow-up data available.



Appendix Figure 2. Graph visualizing the follow up of each ameloblastoma patient together with the mutation status. The X-axis shows the age of the patient at the time of primary detection and the Y-axis shows the follow-up time-line. 33/39 UAM and 21/39 AM cases had follow-up information available. The average follow-up time for the UAMs was 8.2 years and for the AMs and 11,7 years. 14/39 UAMs and 17/21 AMs had one or multiple recurrences, which are marked in the time-lines with colored circles. At the end of the follow-up period, patients without a reported recurrence are marked with a yellow open circle.



Appendix Figure 3. Charts from Kaplan-Meier analyses for comparing recurrence-free survival between UAMs (N=33) and AMs (N=19) as groups and as sub-groups. The two groups were compared in relation to *BRAF* V600V vs. wild-type (charts 1-2), mandible vs. maxilla (charts 3-4), and resection vs. enucleation (charts 5-6). AM and UAM groups were compared (chart 7), the mandibular *BRAF* V600E positive UAM and AM groups (chart 8), and the mandibular AM *BRAF* V600E positive group against the mandibular mural *BRAF* V600E positive UAMs (chart 9). Experiment 1 gave the only statistically significant result (p-value: 0.000346).